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The GMO90+ Project: Absence of Evidence for Biologically Meaningful Effects of Genetically Modified Maize-based Diets on Wistar Rats After 6-Months Feeding Comparative Trial

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1 **Title**

2 The GMO90+ project: absence of evidence for biologically meaningful effects of genetically
3 modified maize based-diets on Wistar rats after 6-months feeding comparative trial

4

5

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50

51

52 **Authors' contributions:**

53 Designed research: XC, RS, BJ, LD, EJ, JPC, NS, FF, FRF, RB, PR, BS

54 Performed research: LJ, YKA, LB, CN, MLM, CDL, CC, JC, SB

55 Analyzed data: XC, RS, LF, YL, JPA, BJ, MTF, CLG, JL, PAG, KDA, NA, BC, AM, BL, BS

56 Wrote the paper: XC, BS

57

58 **Abstract**

59 The GMO90+ project was designed to identify biomarkers of exposure or health effects in Wistar
60 Han RCC rats exposed in their diet to two genetically-modified plants (GMP) and assess additional
61 information with the use of metabolomic and transcriptomic techniques. Rats were fed for six-
62 months with 8 maize-based diets at 33% that comprised either MON810 (11% and 33%) or NK603
63 grains (11% and 33 % with or without glyphosate treatment) or their corresponding near-isogenic
64 controls. Extensive chemical and targeted analyses undertaken to assess each diet demonstrated
65 that they could be used for the feeding trial. Rats were necropsied after three and six months.
66 Based upon the OECD test guideline 408, the parameters tested showed a limited number of
67 significant differences in pairwise comparisons, very few concerning GMP versus non-GMP. In
68 such cases, no biological relevance could be established owing to the absence of difference in
69 biologically linked variables, dose-response effects or clinical disorders. No alteration of the
70 reproduction function and kidney physiology was found. Metabolomics analyses on fluids (blood,
71 urine) were performed after 3, 4.5 and 6 months. Transcriptomics analyses on organs (liver, kidney)
72 were performed after 3 and 6 months. Again, among the significant differences in pairwise
73 comparisons, no GMP effect was observed in contrast to that of maize variety and culture site.
74 Indeed, based on transcriptomic and metabolomic data, we could differentiate MON-based diets to
75 NK-based diets. In conclusion, using this experimental design, no biomarkers of adverse health
76 effect could be attributed to the consumption of GMP diets in comparison with the consumption of
77 their near-isogenic non-GMP controls.

78

79

80 **Introduction**

81 The detection of potential toxicological effects of single chemical compounds tested *in vivo* is
82 generally based on a 90-day (T90) rodent trial to assess any potential unintended effects. The
83 OECD (Organization for Economic Cooperation and Development) 90-day rodent toxicity test has
84 been adapted to food and feed toxicological effects aiming to establish whether genetically
85 modified- (GM) based feed is as safe as its non-GM counterpart (EFSA GMO Panel Working Group
86 on Animal Feeding Trials, 2008; EFSA Panel on Genetically Modified Organisms (GMO), 2011;

87 EFSA Scientific Committee, 2011; European Food Safety Authority, 2014; Devos *et al.*, 2016). A
88 genetically modified organism (GMO) is an individual whose genome has been modified by
89 recombinant DNA technology (genetic engineering) to enhance its performance in a stressful
90 environment or to produce molecules of high economic value. GMOs are now widely used for
91 therapeutic applications, research purposes and with plants (GMP or genetically-modified plants)
92 in the production of feed and other goods. Within the required data for the toxicological assessment
93 of GM plants intended to be placed on the European market (regulation 503/2013 on applications
94 for authorization of genetically modified food and feed in accordance with regulation 1829/2003), a
95 90-day feeding study in rodents on whole GM food/feed to identify potential adverse effects or
96 address remaining uncertainties is mandatory.

97 Despite a large body of evidence pointing to the absence of clinical effects or histopathological
98 abnormalities in organs or tissues of animals fed with GM-based maize (Bartholomaeus *et al.*,
99 2013; Domingo, 2016; Snell *et al.*, 2012), there has been considerable debate recently among
100 public researchers, risk assessment bodies, industry and non-governmental organizations and the
101 public at large (Séralini *et al.*, 2007; Meyer and Hilbeck, 2013; Panchin, 2013; Hilbeck *et al.*, 2015;
102 Antoniou and Robinson, 2017).

103 In an attempt to clarify the issue, the GMO90+ (Genetic Modified Organisms 90-day rodent trial
104 extended to 180-day) project was set up and supported financially by the French Ministry for an
105 Ecological and Solidary Transition. The GMO90+ project gathered expertise from public and private
106 laboratories with the rodent feeding trial conducted under good laboratory practice (GLP) in a
107 contract research organization (CRO). The study sought to provide additional arguments in
108 response to several questions.

109 Firstly, since the 90-day sub-chronic rodent feeding study according to OECD guideline 408 and
110 EFSA guidance has been questioned (Hilbeck *et al.*, 2015), we extended the animal
111 experimentation to 6 months (T180) to establish a putative health effect after 3 months (T190). In
112 addition, 1-year and 2-year complementary experiments in Wistar rats were undertaken at the
113 same time, respectively by the GRACE (<http://www.grace-fp7.eu/>) and G-TwYST ([https://www.g-
114 twyst.eu/](https://www.g-twyst.eu/)) EC funded programs (Schiemann *et al.*, 2014).

115 Secondly, we cultivated two different maize GM varieties and their corresponding near-isogenic
116 counterparts to compare the effect between a Roundup-tolerant and an insect-resistant GM variety
117 chosen from the recent reports and the ongoing EC projects. NK603 maize tolerant to glyphosate,
118 the active herbicide agent in the Roundup® formulation, expresses a bacterial 5-
119 enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, the product of which is not
120 competitively inhibited by the herbicide. MON810 maize resistant to insects expresses a Cry protein
121 complex of *Bacillus thuringiensis*, a larvicidal toxin able to kill lepidopteran pests (Koch *et al.*, 2015).
122 Thirdly, in addition to the classical toxicological approach according to OECD guideline 408, the
123 physiology of kidney, liver and gonads was addressed by detailed analysis including histopathology,
124 biochemistry and hormone quantification to investigate the potential occurrence of alterations in the
125 physiology of these organs as suggested by previous reports (de Vendômois *et al.*, 2009; Séralini *et al.*,
126 2014).

127 Fourthly, to obtain better insights into a potential effect of GM food on rats, we performed omics
128 experiments on different samples from the same rats. Omics analyses used to investigate
129 metabolic variations associated with genetic modifications in the maize grains (Barros *et al.*, 2010;
130 Bernillon *et al.*, 2018; Manetti *et al.*, 2006; Zolla *et al.*, 2008) were only recently assessed to
131 evaluate the impact of GM diet on rat health (Mesnage *et al.*, 2017; Cao *et al.*, 2011; Sharbati *et*
132 *al.*, 2017). In addition, multi-omics analyses were undertaken to discover biomarkers of exposure
133 or effect. Indeed, we also compared the omics data sets to those obtained from clinical parameters
134 (clinical signs, blood and urine assays, organ histopathology). Since we targeted molecular
135 biomarkers, we combined the characterization of global gene expression of two major detoxication
136 organs (liver and kidney) by the determination of the transcriptomes and in parallel, metabolomics
137 on blood and urine samples which could indicate changes of their metabolic signatures. This multi-
138 omics approach is required to assess the multiple phenotypic level of the potential biological
139 consequences of diets that include GM maize. We report the combined results of the toxicological
140 analyses of rats fed with 8 different diets and the multi-omics multi-organs comparisons in a double-
141 blind feeding trial and discuss the biological relevance of the differences observed.

142

143

144 **Materials and methods**

145 ***Maize and diet production***

146 The two varieties harboring the GM maize events MON810 and NK603 were produced under
147 conditions of good agricultural practice jointly with the G-TwYST project in order to cultivate each
148 event at two different geographical sites and thereby overcome production hazards. MON 810
149 (DKC6667YG) and its near-isogenic control (DKC6666) were cultivated at two sites in Catalonia
150 (Spain) along with Sy-Nepal, a conventional variety, used as acclimation diet. NK603 and near-
151 isogenic varieties (Pioneer 8906R and 8906; Prairie Brand 882RR and 882) were cultivated
152 respectively in Ontario (Canada) and Minnesota (USA). Production rules, pesticide treatments and
153 the characterization of the harvests have been reported elsewhere (Chereau *et al.*, 2018) and
154 provided the basis for the choice between the two production sites jointly made with G-TwYST
155 colleagues. Each diet contained 33% maize grains, either of a single genotype or mixed between
156 genotypes as indicated in **Table 1**. An acclimation (ACCLI) diet (33% Sy-Nepal) was produced to
157 feed all animals for two weeks before starting the experiment. This diet did not contain any GM
158 ingredient and had the same composition as the test diets (i.e. maize, wheat and/or coproduct,
159 soybean meal and soy oil) in order to limit the effects of diet change (Chereau *et al.*, 2018). The
160 formulation of the diets was isoproteic, isocaloric and adjusted to the dietary requirements of the
161 rat strain Wistar Han RCC used in the feeding trials. Pellets were produced by Safe Cie (Augy,
162 France) as follows: maize-grinding, pellet preparation, vacuum packaging under 10 kg per batch
163 and diet-encoding. Each batch was beta-irradiated at 29.2 to 35.8 kGy (Ionisos Cie, Dagneux,
164 France) and then sent to the CRO (CitoxLAB, Evreux, France) in charge of the animal
165 experimentation. Diets were stored at 4°C and different samples sent to Eurofins Cie (Nantes,
166 France), Invivo Labs (Chateau-Thierry, France), SGS Cie (Hamburg, Germany) and INRA
167 laboratories for targeted and non-targeted analyses.

168

169 ***Study plan***

170 The study design was based on the OECD TG408 with modifications in order to reach specific
171 objectives such as the extension up to 180 days and omics analyses of blood, urine and organ
172 samples. A total of 30 Wistar Han RCC rats (same rat strain as the one used by the GRACE and

173 G-TwYST projects) per sex were fed with one of eight different diets (**Table 2**): indeed, three sub-
174 groups were established: sub-group A with 10 rats per sex per diet necropsied after 90-day (T90)
175 feeding period; sub-group B with 12 rats per sex per diet followed for 180-day (T180) and sub-
176 group C with 8 rats per sex per diet, both necropsied at T180. Urine and blood samples were
177 collected at several times during the feeding trial, depending on the sub-group. In addition to
178 conventional necropsy and histology, functional properties of liver, kidney and gonads were tested
179 at T90 or T180 (**supplementary Table 1**). Multi-omics was performed using samples from sub-
180 group A with 10 rats per sex per diet necropsied after a 90-d feeding period (T90) and sub-group
181 B with 12 rats per sex per diet followed with blood and urine sampling at T90, T135, T180
182 necropsied after a 180-d feeding period. Transcriptomics was conducted on liver and kidney
183 samples. Metabolomics was conducted on urine and blood. The number of rats in each sub-group
184 was estimated in such a way that the standardized effect size of 1 standard deviation was detected
185 with a power of 80% (see (EFSA Scientific Committee, 2011)).

186

187 ***Rat housing, feeding and sample collection***

188 Animal experimentation was performed at CiToxLAB (Evreux, France). All the study plans were
189 reviewed by the CiToxLAB France ethical committee to assess compliance with the corresponding
190 authorized project, as defined in the Directive 2010/63/EU. The diets were coded in a double-
191 blinded manner. Wistar Rcc®: WIST, Specific Pathogen-Free rats were from Harlan (USA). Males
192 had a mean body weight of 171 g (range: 133 g to 197 g) and the females had a mean body weight
193 of 136 g (range: 115 g to 161 g). Special care was taken to ensure that all animals were born the
194 same day +/- 1 day. Rats were acclimatized to the study conditions for a period of at least 14 days
195 before the beginning of the treatment period with the ACCLI diet (conventional maize variety SY-
196 Nepal). Animals from each sex were allocated to groups using a computerized randomization
197 procedure and care was taken that differences in mean body weight were less than $\pm 10\%$ between
198 groups (per sex). Each animal was identified by an implanted microchip and they were housed two
199 per cage. Males and females were housed in separate study rooms. The cages were placed
200 vertically per group on the racks. One column without animals separated two groups on a rack. The
201 cages rotated within each group from top to bottom on a weekly basis. Every two weeks, all the

202 racks were moved clockwise around the room, rack by rack. Bacterial and chemical analyses of
203 water were regularly performed by external laboratories. The animal room conditions were as
204 follows: $22 \pm 2^{\circ}\text{C}$ temperature, $50 \pm 20\%$ relative humidity, 12h/12h light/dark cycle (light began at
205 4:00 am until 04:00 pm), 8 to 15 cycles/hour of filtered, non-recycled air ventilation. Each animal
206 was observed once a day to record clinical signs and detailed clinical examinations of all animals
207 were performed once a week.

208 The body weight of each animal was recorded on the first day of the experimental period and then
209 once a week until the end of the study. Food and water consumption were calculated each week
210 except during urine collection as rats spent 5 days in a metabolic cage.

211 To obtain a sufficient volume of urine without any external contamination, rats were trained to eat
212 from 4:00 pm to 8:00 pm for 3 days at the beginning of the night cycle without collection of urine or
213 feces (feeding time: T90, T35 and T180). The collection began with no food available at 8:00 pm
214 until 4:00 pm on day 4 in tubes without thymol crystals and were kept on wet ice.

215 Blood samples were collected from the jugular vein without sedation (sub-group B) or from the
216 abdominal aorta at necropsy in tubes containing K_2EDTA or lithium heparin for hematology or
217 clinical chemistry, respectively. Blood samples did not exceed 12.5% of the total circulating blood
218 volume, the same percentage being used for males and females, and the volume collected did not
219 exceed 3 ml.

220 The following investigations were performed on urine samples: urinalysis (CiToxLAB: determination
221 of qualitative, semi-quantitative and quantitative parameters), hematuria and biochemistry
222 (INSERM U1149, Paris), hormonal assays (LABERCA, Nantes) and omics (INRA Toxalim
223 platforms) (**supplementary Table 1**). In the event of small blood volumes, the order of priority was
224 as follows: omics (Profilomic Cie, Saclay/Gif sur Yvette, France), clinical chemistry and hematology
225 (CiToxLAB France), hormonal assays (INSERM IRSET U1085, Rennes).

226

227 ***Gross necropsy, histopathology and biochemistry***

228 On completion of the feeding period (T90 or T180), after at least 8 hours of food deprivation, all
229 rats were deeply anesthetized by an intraperitoneal injection of sodium pentobarbital, necropsied
230 by exsanguination and submitted to a full macroscopic *post-mortem* examination. The body weight

231 of each animal was recorded before necropsy. The following organs were weighed wet as soon as
232 possible after dissection: brain, heart, kidneys, adrenal glands, liver, pancreas, thymus, thyroid
233 glands, spleen, testis, ventral prostate, seminal vesicles, epididymis, ovaries, uterus, vagina. The
234 paired organs were weighed separately: kidneys, testes, ovaries, epididymes. The ratio of each
235 organ weight to body weight was calculated. Tissue procedure is summarized in **supplementary**
236 **Table 2**. For all studied animals, the tissues were preserved in 10% buffered formalin, except for
237 gut, testes, ovaries, epididymes and tissues collected for genomics, for which several preparations
238 were required.

239 The liver was immediately (less than 5 min) weighed following necropsy and three portions of 20
240 to 25 mg of the left lateral liver lobe were placed in 2 mL cryotubes, frozen in liquid nitrogen and
241 then stored at -80°C until shipment to INSERM U1124 for RNA extraction. One portion of the left
242 lateral liver lobe and right median lobe was preserved in neutral buffered 10% formalin for
243 histopathological evaluation at CiToxLAB.

244 Kidney samples for RNA extraction were treated within 5 min following necropsy. The right quarter
245 of the right kidney was placed in a 2 mL tube, snap-frozen in liquid nitrogen and stored at -80°C
246 until shipment on dry ice to INSERM 1124 unit. One half of the left kidney was preserved in neutral
247 buffered 10% formalin for histopathological evaluation at CiToxLAB. The other half was snap-
248 frozen in liquid nitrogen and stored at -80°C until shipment on dry ice to INSERM 1149 unit for
249 immunohistochemistry. Briefly, frozen 4µm kidney slides were incubated with antibodies coupled
250 with biotin anti-IgA and anti-CD11b diluted at 1/100, for 2h at room temperature, to detect
251 immunoglobulin deposits and immune cell infiltration. Detection was performed using the
252 Vectastain elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Slides were mounted with
253 the Immunomount medium (Thermo Fisher Scientific) and observed with an optical microscope
254 (Leica DM2000).

255 For testes and ovaries, the right one was fixed in modified Davidson medium and prepared in
256 paraffin for histopathological evaluation at CiToxLAB France. The left one was frozen in liquid
257 nitrogen, then kept at -80°C and sent to IRSET-INSERM U1085 for hormonal assays. The right
258 epididymis was fixed for histopathological evaluation at CiToxLAB France. The left one was

259 collected and rapidly frozen in liquid nitrogen and kept at -80°C until shipment to IRSET-INSERM
260 U1085.

261 Testicular extracts were used to measure testosterone concentrations by radioimmunoassay (RIA;
262 IM1087 Beckman Coulter, France). Testes were thawed, weighed and homogenized in DMEM-F12
263 medium by using a Polytron homogenizer (Kinematica, Luzern, Switzerland). Each sample was
264 homogenized with 5 times with 1ml of medium leading to 5 ml of testicular extract. Then, 200µl of
265 sample were first assessed for steroid extraction using 2 ml of ether. After freezing of the aqueous
266 phase at -20°C, the ether phase was transferred into glass tubes and evaporated by placing the
267 tubes in a 37°C water bath, before re-dissolving dried extracts in 200µl of recovery buffer. Then
268 50µl of extracted samples were 1/10 diluted in recovery buffer prior to testosterone measurement.
269 The sensitivity of the testosterone assay was 0.03 ng/ml, the intra-assay coefficient of variation
270 was below or equal to 12% and the inter-assay coefficient of variation was below or equal to 12.9%.

271 Plasma estradiol concentrations were assessed by a radioimmunoassay procedure (RIA;
272 DSL4800, Beckman Coulter, France) following the manufacturer's instructions. The minimum
273 detectable concentrations were 2.2 pg/ml and the intra-assay coefficient of variation was 8.9%.

274 Plasma FSH, LH and inhibin B concentrations were determined using rodent ELISA kits (KA2330,
275 KA2332 and KA 1683 from Abnova for FSH, LH and inhibin B, respectively). All procedures were
276 performed according to the standard protocols supplied with a supplementary lower standard point
277 (0.5 ng/ml) for the FSH experiment.

278 To assess sperm production, epididymis was analyzed according to a previously published
279 procedure (Velez de la Calle *et al.*, 1988). Briefly, frozen epididymis was thawed at room
280 temperature, cut into two fragments, the proximal part corresponding to the caput epididymis and
281 the distal part to the cauda epididymis. Each segment was weighed and homogenized in an NaCl
282 0.15M, triton 0.05% buffer. Five cycles of polytron homogenizer (Kinematica) with 1ml of cold buffer
283 were performed for each sample. The final volume of caput or cauda epididymal homogenate was
284 6 ml. The homogenate was observed under the microscope in a Malassez chamber to count
285 spermatozoa. Two counts per samples were averaged. For the homogenization step as for sperm
286 counting, all samples were processed randomly.

287 All tissues required for microscopic examination were trimmed according to the RITA guidelines,
288 when applicable (Kittel *et al.*, 2004; Morawietz *et al.*, 2004; Ruehl-Fehlert *et al.*, 2003), embedded
289 in paraffin wax, sectioned at a thickness of approximately 4 µm and stained with hematoxylin-eosin.
290 A blinded microscopic examination was carried at CiToxLAB on all tissues listed. Afterwards,
291 groups were unblinded and a peer review was performed on all slides of at least 30% of the animals
292 from the groups containing the highest percentages of genetically modified maize (30% from each
293 subgroup A, B or C), and on an adequate number of slides from identified or potential target organs
294 to confirm that findings recorded by the study pathologist were consistent and accurate.

295

296 ***Hematology and clinical biochemistry***

297 Hematology was carried out at CiToxLAB on an ADVIA 120 hematology analyzer/laser
298 (Siemens) to quantify: erythrocytes (RBC), red blood cell distribution width (RDW), mean cell
299 volume (MCV), packed cell volume (PCV), hemoglobin (HB), mean cell hemoglobin concentration
300 (MCHC), mean cell hemoglobin (MCH), thrombocytes (PLT), leucocytes (WBC), reticulocytes
301 (RTC) and neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), large unstained cells
302 (LUC), and monocytes (M). Clinical biochemistry was carried out at CiToxLAB on an ADVIA 1800
303 blood biochemistry analyzer/selective electrode (Siemens) to quantify: sodium (Na), potassium (K),
304 chloride (Cl), calcium (Ca), inorganic phosphorus (P), glucose (GLU), urea (UREA), bile acids
305 (BIL.AC), creatinine (CREAT), total bilirubin (TOT.BIL), total cholesterol (CHOL), triglycerides
306 (TRIG), alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate
307 aminotransferase (ASAT), gamma-glutamyl transferase (GGT), total proteins (PROT), albumin
308 (ALB), albumin/globulin ratio (A/G).

309

310 ***Urine analyses***

311 Urinalysis performed by CiToxLAB included (i) quantitative measurements by using a Clinitek 500
312 urine analyzer/reflecto-spectrophotometer (Siemens) and a specific gravity refractometer (x 1000),
313 (ii) semi-quantitative measurements: proteins, glucose, ketones, bilirubin, nitrites, hemoglobin,
314 urobilinogen, cytology of sediment by microscopic evaluation and (iii) qualitative parameters:
315 appearance, color.

316 To evaluate kidney function at INSERM 1149, 10 μ L of fresh urine were mounted on a Malassez
317 slide to count the red blood cells (hematuria). Protein, albumin and creatinine concentrations were
318 measured in urine using the AU400 chemistry analyzer (Olympus). Neutrophil gelatinase-
319 associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1) urinary concentrations were
320 determined by ELISA using the corresponding kits (R&D Systems, Abingdon UK). NGAL and KIM-
321 1 are two biomarkers of early kidney dysfunction.

322

323 *Urine steroids*

324 To determine steroid hormones (19 different compounds, n=33 targeted quantifications), urine
325 samples from sub-group B were treated with the following steps: hydrolysis of sulfate and
326 glucuronide conjugates by β -glucuronidase from *Patella vulgata* and arylsulfatase from *Helix*
327 *pomatia*, first purification using solid phase extraction (SPE) on a styrene-divinylbenzene (EnviTM
328 ChromP) copolymer, separation of androgens/progestagens and estrogens using pentane liquid-
329 liquid partitioning, second purification of the two fractions on silica-based SPE (SiOH), additional
330 fractionation using semi-preparative HPLC for the estrogen fraction and derivatization by
331 MSTFA/TMIS/DTE for the androgen and estrogen fractions. The measurements were performed
332 by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS), after electron impact
333 (EI) for androgens and atmospheric pressure chemical ionization (APGC) for estrogens, on latest-
334 generation triple quadrupole instruments (Brucker Scion, Waters Xevo TQS). Two diagnostic
335 signals (SRM transitions) were monitored for each target analyte to provide unambiguous
336 identification. Stable isotope surrogates (²H-labeled compounds) were included for individual
337 recovery correction and quantification according to the isotope dilution method, including 17 β -
338 testosterone-d₃, methyltestosterone-d₃, androstendione-d₃, 5 α -dihydrotestosterone-d₃, etiochola-
339 nolone-d₅, 5 α -androstane-3 α ,17 β -diol-d₃, 5 α -androstane-3 β ,17 β -diol-d₃, 17 β -estradiol-d₃.

340

341 *Urine metabolites*

342 Proton nuclear magnetic resonance (¹H NMR) profiling of urine samples was performed at the
343 Metatoul-Axiom facility (MetaboHUB, French National Infrastructure for Metabolomics) and spectra
344 of samples were recorded using a Bruker Avance III HD Spectrometer (Wissembourg, France)

345 operating at 600 MHz equipped with a 5 mm CPQCI cryoprobe. Five hundred μL of urine samples
346 were mixed with 200 μL of 0.2 M phosphate buffer (pH 7.0) prepared in deuterated water, and then
347 centrifuged at 5500 RPM at 4°C for 15 min, and 600 μL of supernatant were transferred to 5 mm
348 NMR tubes. The ^1H NMR spectra were acquired at 300K using the 1D NOESY experiment with
349 presaturation for water suppression, with a mixing time of 10 ms. A total of 128 transients were
350 collected into 32k data points using a spectral width of 20 ppm, a relaxation delay of 2 s and an
351 acquisition time of 1.36 s. Prior to Fourier transformation, an exponential line broadening function
352 of 0.3 Hz was applied to the FID. All NMR spectra were phased and baseline-corrected, then data
353 were reduced using AMIX (version 3.9 Bruker, Rheinstetten, Germany) to integrate 0.01 ppm wide
354 regions corresponding to the δ 10.0-0.5 ppm region. The δ 6.5-4.5 ppm region, which includes the
355 water and urea resonances, was excluded. A total of 751 NMR buckets were included in the data
356 matrix. To account for differences in sample concentration, each integrated region was normalized
357 to the total spectral area.

358

359 ***Plasma sample preparation and analysis by mass spectrometry***

360 *a. Reagents and chemicals.* All analytical grade reference compounds were from Sigma (Saint
361 Quentin Fallavier, France). The standard mixtures used for the external calibration of the MS
362 instrument were from Thermo Fisher Scientific (Courtaboeuf, France). LC-MS grade water (H_2O),
363 methanol (MeOH) and acetonitrile (ACN) was from SDS VWR International (Plainview, NY) and
364 formic acid and ammonium carbonate from Sigma Chemical Co. (St Louis, MO, USA).

365

366 *b. Preparation and analysis sequences.* To limit the degradation of the analytical system
367 performances that occurs during the analysis of a too high number of samples, each time point was
368 subdivided into two batches. Rats raised in the same cage were separated so that each batch
369 contained the same number of males and females and the same number of each (anonymized)
370 diet. To avoid bias due to the sample preparation order and sample analysis order, two different
371 random sequences of samples were used. Stratified sampling was thus performed in each batch
372 using the “sampling” R package (Tillé and Matei, Alina, 2016) to make sure sex and diet were
373 evenly distributed.

374

375 *c. Extraction.* Each plasma sample (50 μ L) was treated with 200 μ L of methanol (MeOH). The
376 resulting samples were then mixed using a vortex mixer for 10 s, left on ice at 4°C for 30 min to
377 allow protein precipitation, then centrifuged for 20 min at 20,000 \times g. Supernatants were dried
378 under nitrogen. Dried samples were then resuspended in 150 μ L of 10 mM of ammonium carbonate
379 (pH 10.5)/ACN, 40/60 (v/v). A quality control (QC) sample consisting of a mixture of equal aliquots
380 of all samples included in this study was injected every five samples. These QC samples were
381 extracted and then injected in triplicate after successive dilutions from 2 to 8 at the beginning of the
382 running sequence after blank series to check the performances of the analytical system and to
383 validate the reliability of the features detected.

384

385 *d. Chromatography.* Experimental settings for metabolomics by LC-HRMS were carried out as
386 previously described (Boudah *et al.*, 2014). Plasma extracts were separated on a HTC PAL-system
387 (CTC Analytics AG, Zwingen, Switzerland) coupled with a Transcend 1250 liquid chromatographic
388 system (ThermoFisher Scientific, Les Ulis, France) using an aSequant ZICpHILIC 5 μ m,
389 2.1 \times 150 mm column (Merck, Darmstadt, Germany) at 15°C. The mobile phase A consisted of an
390 aqueous buffer of 10 mM of ammonium carbonate in water with ammonium hydroxide to adjust
391 basicity to pH 10.5, whereas acetonitrile was used as solvent B. The flow rate was set at
392 200 μ L/min. Elution started with an isocratic step of 2 min at 80 % B, followed by a linear gradient
393 from 80 to 40 % of phase B from 2 to 12 min. The chromatographic system was then rinsed for
394 5 min at 0 % B, and the run ended with an equilibration step of 15 min.

395

396 *e. Mass spectrometry.* After injection of 10 μ L of sample, the column effluent was directly
397 introduced into the heated electrospray (HESI) source of a Q-Exactive mass spectrometer (Thermo
398 Scientific, San Jose, CA) and analysis was performed in both ionization modes. The HESI source
399 parameters were as follows: the spray voltage was set to 3.6 kV and -2.5 kV in positive and
400 negative ionization mode, respectively. The heated capillary was kept at 380°C and the sheath and
401 auxiliary gas flow were set to 60 and 20 (arbitrary units), respectively. Mass spectra were recorded
402 in full-scan MS mode from m/z 85 to m/z 1000 at a mass resolution of 70 k, full width at half-

403 maximum (FWHM) at m/z 200, and by alternating ionization modes. External mass calibration was
404 performed before analysis.

405

406 *f. Identification.* For the putative and the formal identification of endogenous compounds, the
407 metabolite library used in this study was composed of 1000 chemicals available in-house, which
408 includes a wide variety of compounds such as amino acids and their derivatives, carbohydrates,
409 nucleosides, carnitines and derivatives, purines and purine derivatives representing major
410 components of biological matrices (plasma/serum, cerebrospinal fluid, urine and cells). To each of
411 these compounds we also associated their corresponding exact mass, retention time and tandem
412 mass spectrometry data to increase identification confidence. Annotation of the molecules was
413 performed using the software TraceFinder3.3 (ThermoFisher Scientific). It allows the identification
414 of the molecules according to their exact m/z ratio and retention time (RT), but also confirms their
415 identification using a score based on the isotopic pattern. The RT window tolerance and the mass
416 extraction window were set at +/- 0.5 min and 5 ppm respectively. The isotopic pattern was used
417 as a confirmation criterion. The relative isotope abundance (RIA) was evaluated and a score
418 threshold above 80 % was set. The resulting dataset was filtered and cleaned based on quality
419 control (QC) samples as described in Dunn et al. (Dunn *et al.*, 2011): (i) the coefficient of correlation
420 between serial dilutions of QC samples (by factors of 1, 2, 4 and 8) and areas of the related
421 chromatographic peaks should be above 0.8; (ii) the coefficients of variation of the areas of
422 chromatographic peaks of features in QC samples should be less than 30 %; and (iii) the ratio of
423 chromatographic area of biological to blank samples should be above a value of 10.

424

425 *g. Normalization.* To remove analytical drift induced by clogging of the HESI source observed in
426 the course of each batch separately, chromatographic peak areas of each variable were normalized
427 using a low-order nonlinear locally estimated smoothing function (LOESS) fitted to the QC sample
428 data with respect to the order of injection. To remove drift induced by variations in the performance
429 of the analytical system between the two batches of each time point, chromatographic peak areas
430 of each variable were normalized using a ratio calculated between the mean of QC sample data in
431 each batch. The same process was then applied to remove drift induced by variations in the

432 performance of the analytical system between each time point.

433

434 ***Liver and kidney sample preparation and transcriptome analysis***

435 *a. Sample preparation: sampling, total RNA extraction*

436 Before RNA extraction, frozen tissues were submerged in RNAlater®-ICE transition solution (Life
437 Technologies, France) to avoid RNA loss or degradation following the manufacturer's protocol.
438 Afterwards, livers and kidney were placed in 1 mL of Qiazol® reagent with two stainless steel beads
439 (Qiagen, Courtaboeuf, France) and were homogenized with a TissueLyser system (RetschMM300,
440 Germany). Total RNA, including miRNA, was prepared using the miRNeasy Mini Kit according to
441 manufacturer's instructions (Qiagen, Les Ulis, France). The quality of total RNA was monitored with
442 a Nanodrop ND-1000 spectrophotometer (Nanodrop Products, Wilmington, DE, USA) and RIN
443 values were used to evaluate sample quality.

444

445 *b. Microarray gene expression analyses*

446 Gene expression profiles were analyzed at the GeT-TRiX facility (GénoToul, Génopole Toulouse
447 Midi-Pyrénées) using Agilent Sureprint G3 Rat GE v2 microarrays (8x60K, design 074036)
448 according to the manufacturer's instructions. For each sample, cyanine-3 (Cy3)-labeled cRNA was
449 prepared from 200 ng of total RNA using the One-Color Quick Amp Labeling kit (Agilent, Les Ulis,
450 France) according to the manufacturer's instructions, followed by Agencourt RNAClean XP
451 (Agencourt Bioscience Corporation, Beverly, Massachusetts). Dye incorporation and cRNA yield
452 were checked using Dropsense™ 96 UV/VIS droplet reader (Trinean, Belgium). Six hundred ng of
453 Cy3-labelled cRNA were hybridized on the microarray slides according to the manufacturer's
454 instructions. Immediately after washing, the slides were scanned on an Agilent G2505C Microarray
455 Scanner using Agilent Scan Control A.8.5.1 software and fluorescence signals were extracted
456 using Agilent Feature Extraction software v10.10.1.1 with default parameters.

457

458 *c. Microarray miRNA expression analyses*

459 miRNA expression profiles were obtained at the GeT-TRiX facility (GénoToul, Génopole Toulouse
460 Midi-Pyrénées) using Agilent Sureprint G3 Rat v21 miRNA microarrays (8x15K, design 070154)

461 according to the manufacturer's instructions. For each sample, cyanine 3–cytidine bisphosphate
462 (pCp-Cy3)-labeled RNA was prepared from 100 ng of total RNA using miRNA Complete Labeling
463 and Hybridization Kit (Agilent Technologies, Les Ulis, France). The labeled RNA was hybridized on
464 the microarray slides according to the manufacturer's instructions. Immediately after washing, the
465 slides were scanned on an Agilent G2505C Microarray Scanner using Agilent Scan Control A.8.5.1
466 software and fluorescence signals extracted using Agilent Feature Extraction software v10.10.1.1
467 with default parameters.

468

469 **Statistics**

470 A global methodology was used for all the analyses and datasets with some differences related to
471 the specificities of each dataset in order to facilitate the interpretation of the very large amount of
472 data generated. The rat and not the cage, was taken as the experimental unit, except for the feed
473 and water consumption for which we only had a measurement per cage. The two sexes were
474 analyzed separately, and all the statistical tests were performed with a type I error of 5% with a
475 false discovery rate correction (FDR) (Benjamini and Hochberg, 1995) for large datasets.

476

477 *a. Datasets from toxicological experiments*

478 First, for each endpoint, research of potential outliers is performed visually and by a statistical
479 procedure (Grubbs, 1950). Then, experts decided to include or exclude these potential outliers
480 based on biological plausibility. Most of the identified values were included in the study (only
481 between 0 and 0.2% of each dataset were excluded).

482 Second, a blind analysis was carried out to assess the eventual effect of the eight diets without any
483 a priori assumption. Any differences due to the global variability of the diets for each endpoint could
484 therefore be established. The differences between the diets were tested with one-way analysis of
485 variance (ANOVA) when all the required assumptions were met. Otherwise, the non-parametric
486 Kruskal-Wallis (KW) test was used. In the event of a significant result, differences between diets
487 were examined pairwise by applying post-hoc tests such as Dunnett or Nemenyi tests to highlight
488 the statistical differences. The biological or toxicological relevance of statistically significant
489 differences was considered a matter of expert judgement. Contrary to the GRACE project (Schmidt

490 *et al.*, 2016), we did not apply equivalence tests for two reasons: (i) our CRO does not have any
491 historical data for this kind of study; (ii) equivalence testing has not yet been developed for omics
492 data and we wanted to use a similar global approach to analyze all our datasets.

493 Feed and water consumption and weight measurements were analyzed using mixed effect models
494 (Laird and Ware, 1982; Davidian and Giltinan, 1995). These models allow a comprehensive
495 analysis of longitudinal repeated measurements as explained in Schmidt *et al.* with a focus on
496 linear models (Schmidt *et al.*, 2016). According to the graphical representations of the raw datasets,
497 the weight measurements were modeled using the non-linear Mitscherlich model, as described
498 by the ANSES guidelines (2011), whereas a linear model was applied to the feed and water
499 consumption (Anses, 2011). The diets were considered as a fixed factor and their potential
500 influence was tested using a likelihood ratio test.

501

502 *b- Datasets from omics experiments*

503 (i) *Common global approach*

504 First, a blind analysis was carried out by independent entity, including the eight diets, to assess the
505 eventual effect of the diets without any *a priori* on the diets. This approach makes it possible to
506 investigate whether there is a difference due to the global variability of the eight diets at each
507 endpoint. A first descriptive method reducing the dimensionality of the various analyses was used,
508 Principal Component Analysis (PCA), to detect the first global trends contained in each dataset
509 (Jolliffe, 2002). The statistical relevance of the differences between the diets was then assessed
510 using ANOVA or Kruskal-Wallis test and the corresponding post-hoc tests. The biological or
511 toxicological relevance of significant differences was considered a matter of expert judgement.
512 Second, heuristic relevant pairwise comparisons were carried out to answer our main objective
513 identification of biomarkers of exposure and potentially of effect that are GMO or glyphosate
514 dependent, according to three targeted scientific questions. The GMO effect that could be checked
515 either with MON810 or NK603 was based on the following comparisons: ISOMON versus MON11
516 or MON33 and MON11 versus MON33; ISONK versus NK11 or NK33 and NK11 versus NK33. The
517 glyphosate effect, which would be indirect since all diets contained similar low contents, was based
518 on the comparison between NK11 or NK33 versus NKG11 or NKG33, respectively. We also tested

519 the combined effect of variety and environment effect, since each type of maize (MON810 or
520 NK603) was cultivated in different environmental conditions (Spain and Canada), namely the
521 comparisons between ISOMON versus ISONK, MON11 versus NK11 and MON33 versus NK33.
522 Obviously, the results of all these targeted comparisons had to be carefully analyzed jointly with
523 the global analysis results. These targeted comparisons were performed using the same statistical
524 methods detailed above (PCA, ANOVA ...). Partial Least-Square Discriminant Analyses (PLS-DA)
525 was used to extract from a dataset with a high number of variables the ones that best differentiate
526 the diets, i.e. the variables that are the most different among the diets. For more details on this
527 method, the interested reader is referred to (Frank and Friedman, 1993). The number of
528 components was determined using K-fold cross-validation (10-fold). When different times of sample
529 collection were available, a joint analysis that combines information available at all timepoints was
530 also carried out.

531

532 *(ii) Details for each dataset*

533 *Microarray data.* Raw data (median signal intensity) were filtered, log₂ transformed, summarized
534 to probe level, corrected for batch effects (microarray washing bath serials) and normalized using
535 quantile method (Bolstad *et al.*, 2003). Raw data were also summarized to mRNA level. A model
536 was fitted using the limma lmFit function (Smyth, 2004). Pair-wise comparisons between biological
537 conditions were applied using specific contrasts. A correction for multiple testing was applied using
538 the FDR, probes with $FDR \leq 0.05$ were considered to be differentially expressed between
539 conditions. Statistical analyses were performed using R (R Core Team, 2008) and Bioconductor
540 packages (Gentleman *et al.*, 2004).

541

542 *Plasma.* Statistical analyses of plasma data were performed by sex independently for each time of
543 the study (T90, T135 and T180). Then a joint analysis including all time-points was carried out.
544 Logarithm transformation was applied to the data and P-values were corrected using FDR. In the
545 case of significant results, differences between diets were examined pairwise applying post-hoc
546 tests: Tukey's tests for normal distributions, Nemenyi's otherwise. Logarithm transformation was
547 applied to the data, Transformed data were then centered and reduced. For the differential

548 analyses, the number of components was determined using K-fold cross validation (10-folds). The
549 joint analysis combined information available at all time-points. Differential analysis on all study
550 time-points was performed thanks to a mixed effect model including diet, time and their interaction
551 as fixed effects, and rat as a random effect. P-values were corrected using the FDR. PLS-DA was
552 also carried out taking into account all study time-points thanks to the “multilevel” option of the
553 mixOmics package. All analyses were performed with R software version 3.2.2 (nlme and
554 mixOmics packages, mixomics.org).

555

556 *Urines*. SIMCA-P software (V14, Umetrics AB, Umea, Sweden) was used to perform the
557 multivariate analyses of ¹H NMR profile data. R software was used to perform the univariate
558 analyses. Significant NMR variables were identified using 1D and 2D NMR spectra of in-house
559 libraries and spectral databases (HMDB, www.hmdb.org).

560 For each dataset, a statistical analysis plan was written and validated before the analyses. If any
561 modifications were made, they were reported on a new version of the plan. All the datasets are
562 stored in the website CADIMA (Central Access Database for the Impact Assessment,
563 <https://www.cadima.info/index.php>) under the administration of the Julius Kühn Institute
564 (Quedlinburg, GER), so interested readers can reproduce the findings.

565

566 ***Dialog body***

567 The GMO90+ project took place in a context where societal debate on the environmental and health
568 impact of GM organisms was highly controversial. Consequently, a dialog body was organized by
569 Anses (French Agency for Food, Environmental and Occupational Health & Safety) to involve
570 stakeholders in the development of the project. The expected objectives were as follows: (i) collect
571 the questions and expectations of different stakeholders in civil society, (ii) foster conditions for
572 mutual understanding of the objectives and conditions of the research project, (iii) mobilize all
573 existing data or knowledge in order to enrich the research content and approach, (iv) identify the
574 objects and possible points of controversy on which it was important to be particularly vigilant when
575 conducting the research protocol. The composition was finalized after a public call for expression
576 of interest targeting all representative associations, companies and organizations (including non-

577 governmental organizations) with activities and/or knowledge in the field of GM plants and their
578 toxicological analysis. The first meeting of the dialog body was organized on May 28, 2014. During
579 this meeting, almost all the representatives of the NGOs expressed their decision to withdraw from
580 the dialog body, notably for reasons related to the modalities of the research project itself and the
581 participation of representatives from industries (verbatim of the meeting, [http://recherche-
582 riskogm.fr/sites/default/files/projets/verbatim_instance_dialogue.pdf](http://recherche-riskogm.fr/sites/default/files/projets/verbatim_instance_dialogue.pdf)). Consequently, to replace the
583 dialog body, a communication committee was set up with representatives from INRA, Anses,
584 INSERM and the Ministry for an Ecological and Solidary Transition to update some news on a
585 website dedicated to the project (<http://recherche-riskogm.fr/en/page/gmo90plus>). The key points
586 of the GMO90+ project were presented in 2015 during two Anses "Thematic steering committees"
587 open to the stakeholders.

588

589

590 **Results**

591 ***Diet composition analysis***

592 Maize culture, harvest, chemical and genetic analyses are reported elsewhere (Bernillon *et al.*,
593 2018; Chereau *et al.*, 2018). NK 603 (NK) and MON 810 (MON) diets were detected at expected
594 levels for their genetic traits but genetic analysis showed traces (between 0.1% and 0.2 %) of
595 unexpected GMO events in the ISONK, NK11 and ISOMON diets. The biochemical composition of
596 the grains was characterized by using targeted analyses and metabolomics profiling (Bernillon *et al.*
597 *et al.*, 2018). The chemical composition of the diets (**supplementary Table 3**) showed that a few
598 parameters were slightly below the nutritional reference values (Nutrition, 1995) which should not
599 raise concern over their potential metabolic disturbances in rats: methionine (minus 5% for NKG33
600 and ISONK), threonine (minus 3% for NK11, ISONK and MON11), pyridoxine (all diets below 6
601 mg/kg), vitamin B12 (lower values for NK11, ISONK, ISOMON). All the diets were slightly
602 contaminated by glyphosate at about the same level, globally less than 75 µg/kg, which is far below
603 the maximum residue level (MRL) of 1000 µg/kg. This was due to non-GM soybean that contained
604 residues of glyphosate and its main metabolite, aminomethyl phosphonic acid AMPA (mean 3.3
605 and 5.7 mg/kg, respectively). Consequently, a glyphosate effect can only be tested as an indirect

606 effect on maize composition not as a potentially disrupting component of the pellets for NKG diets.
607 A careful and complete analysis of a large set of over 1000 genetic and biochemical parameters
608 showed slight differences for 15 of them between diets and mainly between the two groups of diets,
609 MON- and NK-based diets (Chereau *et al.*, 2018). In conclusion, this large set of analyses
610 demonstrated that the eight types of diets fulfill the nutritional requirements for Wistar rats and
611 contain traces of undesirable substances that do not raise safety concerns for them and would not
612 interfere with the results of the study.

613

614 ***Feed consumption and body weight***

615 No statistical effect of the diets was observed on the body weight of males or females. The modeling
616 of each condition using non-linear Mitscherlich mixed models is shown in **supplementary Figs.1a**
617 **and b**. Regarding feed and water consumption, there was no statistical effect of the diets for male
618 and female respectively. **Supplementary Figs.1c and d** show the modeling using linear mixed
619 models of feed consumption.

620

621 ***Clinical observations***

622 Daily and weekly observations showed that a few rats of both sexes presented minor clinical signs,
623 most of them in sub-group B and for both sexes (**supplementary Tables 4a-b**). A few animals in
624 almost all groups occasionally presented abnormal growth of teeth, chromodacryorrhea, scabs,
625 nodosities, thinning of hair or soiling. This was considered to be part of the normal background of
626 this strain in view of their low incidence. No clinical signs indicative of systemic toxicity was noted
627 in any animals. There was no dietary effect between GM varieties or between GM maize compared
628 to its near-isogenic control with regard to the frequency of appearance of clinical effects. Only one
629 rat (female E25047, subgroup B, diet NKG33) out of 480 that showed signs of poor clinical condition
630 was humanely killed for ethical concern on day 118.

631

632 ***Hematology and clinical biochemistry***

633 To reach the minimum statistical power of 80%, results were pooled for rats from sub-groups A and
634 B at T90 and for rats from sub-groups B and C at T180, i.e. at least 20 rats per sex per experimental

635 time and per diet. A blind analysis was carried out to assess the potential effect of the diets without
636 any a priori on the diets. The 28 comparisons at T180 showed few significant differences such as
637 WBC, PWBC for the males and E (%) for the females. Then an unblinded analysis was conducted
638 with 14 comparisons to check for a GM effect (six comparisons encoded 1 to 6, see **Table 3**), a
639 combined GM and glyphosate effect (three comparisons encoded 7 to 9), a glyphosate effect (two
640 comparisons encoded 10, 11) and a maize variety effect (three comparisons encoded 12 to 14).
641 As shown in **Table 3a**, six variables measured in the male samples at T180 showed differences,
642 most of them occurring with one diet comparison only, generally the maize variety and environment
643 effect. Similarly, the female samples showed 10 variables with significantly different values, most
644 of them occurring with one diet comparison, generally the maize variety and environment effect
645 (**Table 3b**). The comparison between the results obtained with the males and the females showed
646 only two correspondences with eosinophils (NK11 versus NKG11) and WBC (MON11 versus
647 NK11). The hematologic profile of T90 and T135 samples from the males and females reported
648 respectively in **supplementary Tables 5a and b** showed an absence of correlation in the
649 statistically different variables during the experimental time between samples of each sex and
650 between sexes.

651 Clinical biochemistry values were not significantly different in males but Na, CREA and ALAT
652 values significantly differed between diets for female plasma at T180 (data not shown). Results at
653 T90 showed significant values in Ca, P (phosphate) and ALB for male plasma and in the A/G ratio
654 for female plasma. An unblinded analysis showed differences in eight variables from male samples
655 at T180, most of them occurring with one pair of diets and generally corresponding to the maize
656 variety and environment effect (**Table 4a**). Similarly, 11 variables showed differences in female
657 samples, most of them occurring with one pair of diets and generally corresponding to the maize
658 variety and environment effect (**Table 4b**).

659 The results at T90 and T135 shown in **supplementary Tables 6a and b** indicate that most of the
660 statistically significant differences between the diet groups were related neither to the experimental
661 time (T90 vs T135 vs T180) nor to the concentration of GM-feed in the diet (0%, 11%, 33%), but
662 were mostly related to the maize variety.

663

664 ***Relative organ weight, gross necropsy and histopathology***

665 Concerning pathology, differences in organ weights between diet groups were minor as reported
666 in **supplementary Tables 7a and b**. Statistically different values of relative weight were observed
667 in males at T180 in the case of kidney, pancreas, thymus, thyroid, right testis, ventral prostate,
668 seminal vesicles and left epididymis and in the case of seminal vesicle at T90 (**supplementary**
669 **Table 7a**). The differences were mostly related to consumption of MON compared to NK diets.
670 There was only one difference for the female organs: right ovary at T180 (**supplementary Table**
671 **7b**). Macroscopic findings at necropsy did not reveal any relevant abnormalities (**supplementary**
672 **Tables 8a and b**). In addition, the number of macroscopic differences in sub-groups B and C (T180)
673 compared to sub-group A (T90) did not increase. Macroscopic differences in organs were minor
674 and typical of animals of this strain and age between groups. No clinically relevant abnormalities
675 were found.

676 Microscopic histopathologic analysis was conducted blind, so the scoring led to a higher level of
677 background noise than in the case of a pairwise comparative analysis. As illustrated in
678 **supplementary Tables 9a-b**, only minor microscopic observations were identified with the
679 exception of the female reproduction tract with cysts in the ovaries more frequent at T180 than at
680 T90. However, this is known to be typical of this animal strain and age. No malignant tumor was
681 detected in any sex or group. There was no increase in the frequency of microscopic abnormalities
682 in the sub-groups B and C compared to sub-group A. Indeed, the few abnormalities in animals fed
683 with the different diets as revealed by histopathological analyses occurred to a similar extent in
684 each sub-group.

685

686 ***Urinalysis and kidney physiology***

687 Quantitative urinalysis (**supplementary Tables 10a-b**) showed very few significant differences
688 between diets. Kidney physiology was studied by quantifying proteinuria, albuminuria and
689 hematuria. As shown in **supplementary Fig. 2**, the value of these parameters did not differ
690 between diets at T180. Results were similar for urine samples from the same sub-group B at T90
691 (data not shown). In addition to the microscopic examination that did not show any inflammatory
692 process (immunohistochemistry, data not shown), the concentration of two biomarkers of early

693 kidney dysfunction, NGAL (**supplementary Fig. 3a-d**) and KIM1 (**supplementary Fig. 3e-h**) was
694 quantified in urine. Again, there was no difference whatever the sex or diet, nor was there any
695 difference at T135 (data not shown). Therefore, renal function was not compromised in any group
696 and protein markers were normal in all urine samples.

697

698 ***Reproductive system***

699 The following determinations were carried out to test the potential effect of the diets on the
700 reproductive system: (i) organ weight, (ii) plasma hormone levels with additional parameters in
701 males such as accessory gland weights and intra-testicular testosterone levels and (iii) epididymal
702 sperm reserves. To characterize potential disruptions induced by the diets at the steroidogenic
703 level, endogenous steroid hormones at trace levels in urine samples were also sought by targeted
704 MS analyses. In males, epididymal sperm reserves in both the caput and cauda at T90 and T180
705 were as expected and did not show any difference between the diet groups (**supplementary Fig.**
706 **4**). The concentration of intra-testicular testosterone displayed large variations (Fig. 1a-b) but a
707 difference only at T180 with a lower level in rats fed with the MON11 diet than the ISOMON near-
708 isogenic controls (**Fig. 1b**). There was no difference between rats fed with the MON33 diet and
709 those with the ISOMON diet, a group presenting a relatively high testosterone level. The
710 hypothalamus/pituitary/testis axis was analyzed by quantifying plasma testosterone, LH, FSH and
711 inhibin B hormone levels. The slight but significant difference in the levels of intra-testicular
712 testosterone in MON11 compared to ISOMON diet group at T180 was also found for circulating
713 testosterone concentrations (**Fig. 1d**). There was no difference in plasma LH levels between the
714 eight groups (**Fig. 1e-f**). However, to compensate for the large inter-individual variations due to the
715 pulsatility of hormonal production, we also calculated the ratio testosterone/LH. No difference was
716 found between the eight groups (**Fig. 1g-h**). Taken together, these results and the absence of
717 difference between the weight of the accessory glands indicate an absence of effect of the diets on
718 the Leydig cell function and on the LH-testosterone axis. Similarly, there was no difference in
719 plasma FSH levels between the eight diets at T90 (data not shown). However, a slight but
720 significant increase in inhibin B levels in the NK603 GM diet group (NK11, NK33, NKG33 vs ISONK)
721 was observed at T90 for sub-group A (**Fig. 1i**). At T180 (sub-group B), results showed a slight but

722 significant decrease in the NK11 group compared to the NK-G11 group (**Fig. 1j**). There was no
723 difference in the ratio FSH/inhibin B at T90 for sub-group A (data not shown).

724 In females, no ovarian abnormalities were noted between the eight groups (**supplementary Table**
725 **8b**), suggesting an absence of effect of the diets on ovarian function. The
726 hypothalamo/pituitary/ovary axis was analyzed by quantifying estradiol (E2), LH and FSH levels in
727 plasma. E2 levels showed no significant difference between the eight groups at both feeding times
728 (**supplementary Fig. 5a-b**). Likewise, there was no difference in circulating LH (**supplementary**
729 **Fig. 5c-d**) and FSH (**supplementary Fig. 5e-f**) levels between the groups, confirming the integrity
730 of ovarian function in all of them.

731 The urinary steroidome was then investigated in each sex: among the 33 targeted steroid
732 hormones, 19 were detected with significant consistency (i.e. detection rate > 50%) in females,
733 while only 6 and 8 were significantly detected in males at T90 and T180. In male samples collected
734 at T90, the difference between the diet groups was significant only for pregnenolone and
735 progesterone among the six hormones considered (**Table 5**). Globally, a tendency was observed
736 toward higher levels of pregnenolone with concomitant lower levels of progesterone for the
737 ISOMON and MON33 diet groups, with varying statistical significance depending on the groups
738 compared (**Fig. 2a-b**). For samples collected at T180, the difference was significant only for 5 α -
739 androstenedione among the eight hormones considered (**Table 5**). After post-hoc adjustment, this
740 statistical difference remained only for the comparison between the NK33 and ISOMON diet groups
741 (**Fig. 2c**).

742 In female samples collected at T90, a significant difference was observed for two of the 19
743 hormones investigated, namely pregnenolone and 5 α -dihydrotestosterone (**Table 5**). However, the
744 difference remained significant only for 5 α -DHT after an adjusted Mann-Whitney test (**Fig. 3a and**
745 **b**). The tendency previously observed for pregnenolone in males was not observed. For samples
746 collected at T180, there was a significant difference for four of the 19 hormones, namely DHEA, 5-
747 androstene-3 β ,17 β -diol, 17 α OH-progesterone and 5 α -androstane-3 β ,17 α -diol (**Table 5**). After
748 post-hoc adjustment, this statistical difference remained only for DHEA between groups fed ISONK
749 and NK11 diets, and for 17 α OH-progesterone between groups ISOMON and ISONK as well as
750 between groups fed ISOMON and NK-G33 diets (**Fig. 3c, d, e and f**).

751 Altogether, we observed very few differences in terms of the urinary endogenous steroid profile. In
752 addition, these differences are not biologically consistent with regard to the steroidogenesis
753 pathways, their interpretation in physiological terms was impossible despite their statistical
754 significance. In summary, our steroidomic data did not show any significant disruption of the
755 steroidome for males and females exposed to a GM-based diet after a 90-day or 180-d feeding
756 period.

757

758 ***Liver and kidney transcriptome***

759 In order to obtain deeper insight into a putative effect of GM feed in rat physiology, a full
760 transcriptomic (liver and kidney samples) and metabolomic (plasma and urine samples) analysis
761 was conducted. We first performed principal component analyses (PCA) with the liver
762 transcriptome of rats sacrificed 180 days (T180) after the beginning of the GMO-protocol. We
763 observed as expected a clear separation between two groups of individuals perfectly identified by
764 the sex factor (**Fig 4a**). This result is also found with the kidney transcriptome (data not shown).
765 On the contrary, a very low variability due to any other factors such as diet was observed with the
766 results of the liver transcriptome at T180 (PC3 and PC4: 2% of explained variability; **Fig. 4b**). A
767 similar pattern of variability was observed with the data from the liver transcriptome of sub-group A
768 (T90) as with the kidney transcriptome at different time points (data not shown). Overall, this first
769 descriptive exploratory analysis suggested no clustering effect of the different diets; the sex factor
770 was responsible for a high variability in the transcriptomic profiles analyzed. First a global PLS-DA
771 was performed per each sex. In male rats, the results obtained from liver samples at T180 showed
772 a very low variability according to the diets and no clear distinction was observed between them
773 (**Fig. 5a-d**). Results were similar at T90 for liver and at both experimental times for kidney, and
774 overall for the females (data not shown). The next step was to examine a selection of diets for an
775 effect of glyphosate treatment, maize variety in the diet, and GM versus non-GM diet. Since
776 glyphosate levels were similar in all the diets, our experimental plan allowed us only to test the
777 indirect effect of the glyphosate treatment of NK603, i.e. the effect of metabolic changes in kernel
778 composition induced in the growing plants (hereafter termed “glyphosate effect”). In liver at T90,
779 the number of differentially expressed genes (DEGs) was 1 when NK33 vs NKG33 conditions were

780 compared in females (**Table 6**). In kidney, the number of DEGs was very high (3658) but only in
781 males and at T180; this high number was however, not correlated to major metabolic modifications
782 (plasma and urine, see below), neither to the activation of a major signaling pathway. No other
783 difference was observed using the transcriptomic analyses conducted both with mRNA and miRNA.
784 The genetic background of maize (MON or NK) in conjunction with the growing area (Spain for
785 MON or Canada for NK) may lead to different omics signatures. A PLS-DA, which sharpens the
786 separation between groups of observations, shows a clear distinction between both MON810 and
787 NK603 diets (**Fig. 6**). Interestingly, liver transcriptomic analysis revealed the highest number of
788 DEGs when comparing MON versus NK diets. At T90, various genes were differentially expressed
789 significantly in the liver of both sex in the three conditions ISOMON vs ISONK, MON11 vs NK11
790 and MON33 vs NK33 (**supplementary Table 11 and 12**). The number of DEGs was far below the
791 total number of DEGs linked to the sex effect and no biological relevance could be established
792 owing to the lack of information related to these changes or to the lack of difference in biologically
793 linked variables for all comparisons. No DEMI was significant (**Table 6**). When analyzing kidney-
794 related transcriptomics data at T90, we found a lower number of DEGs 0, 0 and 1 in females and
795 1, 3 and 6 in males (**Table 6, supplementary Table 13**). At T180, we found a low number of DEGs
796 in females but not in males (**supplementary Table 14**). No DEMI was significant (**Table 6**).

797 The main objective was to evaluate the effect of GMO versus non-GMO consumption. In female
798 livers, the number of DEGs was only 1 at T90 in MON33 vs ISOMON conditions and 2 at T180 in
799 MON11 vs ISOMON conditions while in male livers, the number of DEGs was 11 at T180 in NK11
800 vs ISONK conditions (**supplementary Table 15**). No other modifications (DEGs, DEMI) were
801 observed in male or female livers. In kidney, the number of DEGs was very low (1) at T90 when
802 comparing NK11 vs ISONK and NK33 vs ISONK in females. In male kidneys, the number of DEGs
803 was quite high (822 and 563) at T180 in MON33 vs ISOMON and MON33 vs MON11 conditions,
804 but remarkably low (2 and 1) when comparing NK33 vs ISONK conditions at T90 and T180 (**Table**
805 **6, supplementary Table 16**). The number of DEMI was low (3 and 1) in MON33 vs ISOMON and
806 NK33 vs NK11 conditions in males (**supplementary Table 16**).

807

808 ***Urine and plasma metabolomic***

809 The results from the principal component analyses (PCA) with the data generated by urine or blood
810 metabolome was similar to that observed with liver and kidney transcriptome showing a clear sex
811 effect (data not shown). A PLS-DA analysis on metabolomic data was performed per each sex
812 **(Fig. 7)**. The results showed a very low variability according to the diets and no clear distinction
813 was observed between them (data not shown).

814 Then, the indirect glyphosate effect was tested using the plasma or urine metabolomics data at
815 T90, T135 and T180 **(Table 7)**. Concerning the targeted metabolomic analysis of plasma samples,
816 a blinded statistical analysis of the diet effect was performed on the 280 different molecules
817 identified out of the 1000 compounds of the databank. There was no change in metabolite
818 concentrations when the diets were compared by pairs. Furthermore, unblinded data from blood
819 samples did not reveal a glyphosate effect in either sex at any of the three timepoints. Concerning
820 urine, the four diets could be discriminated but PLS-DA models were not valid. Based on PLS-DA,
821 the diets could be discriminated in both sex groups and at the three timepoints except at T135
822 (NK11 vs NKG11) for males **(Table 7)**. As many as five metabolites were found to discriminate the
823 diets **(supplementary Tables 17-19)**. However, if we discard the result when either the level of
824 one metabolite was increased at a timepoint but decreased at another one or when the variation
825 was found only at one timepoint, only taurine and indoxylsulfate were discriminant. Indeed, there
826 was a slight decrease (>0.65) in taurine with the NKG diets at T90, T135 and T180 in females and
827 a slight increase (<1.3) in indoxylsulfate at T90 and T135 in males.

828 Similarly to the transcriptomic analysis, we observed a maize variety and environment effect. For
829 plasma, a blinded analysis performed per sex showed a clear effect since the concentrations of 9
830 and 13 metabolites were significantly altered in males and females, respectively, which had
831 consumed the ISOMON, MON11 or MON33 diets in comparison with the other diets. Then,
832 unblinded data allowed discriminating the MON and NK diets on the basis of 8, 3 and 15
833 metabolites in males and 0, 1 and 3 metabolites in females, at T90, T135 and T180 respectively
834 **(Table 7)**. The diets could be discriminated by a few plasma metabolites repeatedly found at slightly
835 different concentrations: stachydrine, methionine sulfoxide, 2-ceto-4-methylthiobutyric acid
836 (KMBA) and 1-5 anhydro-d-sorbitol in males ($0.97 < \text{ratios} < 1.03$) and stachydrine, ergothioneine,
837 eugenol and 2-furoic acid in females ($0.97 < \text{ratios} < 1.03$) **(supplementary Table 20)**. For urine, a

838 clear discrimination was also found between the three diet pairs. For example, the comparison
839 between ISOMON vs ISONK, except at T90 in females, provided a model with a high level of
840 prediction (2 elements, R^2 close to 100%, Q^2 close to 1). Similar results were obtained for both sex
841 at all timepoints when comparing MON11 versus NK11 and MON33 versus NK33. PLS-DA showed
842 that at T90, T135 and T180 and in both sexes except females at T90, the concentrations of 2-20
843 metabolites in urine were significantly altered (**Table 7, supplementary Tables 17-19**). The
844 difference in metabolite levels was more pronounced with NK11 vs MON11 and NK33 vs MON33
845 than with ISONK vs ISOMON, as was the case for the female urine compared to the male urine.
846 Considering the same prerequisites as above, the significantly altered metabolites evidenced by
847 comparing NK versus MON diets are shown in **supplementary Tables 17-19**. However, only the
848 concentrations of cis-aconitic acid, pantothenic acid and trigonelline were modified more than once
849 when comparing the three diets at the three timepoints for males and 2-oxoglutaric acid for both
850 males and females. In conclusion, omics data indicated that the MON diets were clearly different
851 from the NK ones, the best separation being obtained with metabolites quantified in urine.

852 We then studied the GM-effect with MON- and NK-based diets. For plasma, no modification was
853 identified (**Table 7**). For urine, the model was valid for both females or males, except at T180. The
854 MON GM versus non-GMdiets could be discriminated (4 or 5 elements, R^2 close to 95%, Q^2 close
855 to 0.8) but the models were less predictive than those testing the effect of maize genetic
856 background. PLS-DA obtained at T90, 135 and 180 showed few alterations in the metabolites
857 (**Table 7, supplementary Tables 17-19**). Using the same prerequisite as mentioned above, only
858 the concentrations of glucuronic acid and indoxylsulfate were found to be altered in urine when the
859 pairs of MON diets were compared in females, while only citric acid was modified in males. In the
860 case of NK-based diets, the models were not valid for females at T135 and T180 or for males at
861 T90. The differentiation between the NK GM versus non-GM diets was obtained with a low level of
862 prediction (Q^2 about 0.6). PLS-DAs indicated several modifications that had occurred both in males
863 and females at the three timepoints (except for one out of 18 comparisons) and with all the diets.
864 The modifications were more pronounced in females than in males. However, using the
865 prerequisite mentioned above, only dimethylamine and glycocholic acid were altered in females

866 and 2-oxoglutaric acid in males. The differential concentrations of these metabolites in urine could
867 not be correlated with any pathophysiological signature.

868

869

870 **Discussion**

871 The GMO90+ sub-chronic feeding trial was designed to fulfill multiple goals by combining a
872 classical toxicology study following the OECD TG 408 guidelines (with modifications) and omics
873 approach. To our knowledge, this is the first time that MON810 and NK603 maize-based diets were
874 used side by side to feed rats for 6 months in order to analyze potential GMP effects on the basis
875 of a large number of parameters including pathologic to molecular markers. In line with
876 transparency in research activity on the health impact of GMP, a dialog body has tentatively been
877 set up to promote public participation as a way to increase trust in institutional practices. The
878 causes of the failure of operating this instance is currently being analyzed and the conclusions will
879 be reported elsewhere. The quality and equivalence of the different diets was ensured by a
880 complete compositional analysis in which a large number of molecules were detected and
881 quantified by targeted analyses (Chereau *et al.*, 2018). The nutrient and anti-nutrient composition
882 of the diets showed no substantial differences as with contaminants between GM and non-GM or
883 NK and NKG pellets. The data for glyphosate were surprising. Indeed, all diets were found to
884 contain glyphosate, with slightly higher levels in NKG-based pellets, while glyphosate had only
885 been detected in NKG grains. It is likely while unexpected that the presence of glyphosate residues
886 in all diets results from a weak contamination of the organic soybeans culture from South America.
887 Consequently, a “glyphosate effect” can only be tested as an indirect effect on maize composition
888 not on rat health. This was referred to as the “glyphosate effect” throughout this study. However,
889 two differences were observed with fumonisins and lead contaminants present at higher level
890 although at nontoxic dose, in MON- by comparison with NK-based diets. In addition to classical
891 targeted biochemical analyses, we also performed metabolomic analyses of the pellets (Bernillon
892 *et al.*, 2018). Again, the great majority of statistically significant differences in composition of the
893 pellets, was attributed to the combined effect of variety and environment. In comparison, transgene
894 and glyphosate effects remained limited in grain and pellet for the compound families studied.

895 The rat body weight was measured for 3 months (sub-group A) and 6 months (sub-groups B and
896 C) feeding trial. There was no significant difference between the diet groups either for feed
897 consumption or body weight of rats of both sexes and consequently no GM effect both with MON
898 and NK-based diets. A limited number of minor clinical signs were observed mostly in rats of both
899 sexes from sub-group B which manifested about 66 % of clinical signs, whereas sub-groups A and
900 C manifested about 33%. This might have been due to a higher level of stress in this sub-group
901 which underwent blood tests on the jugular vein (times T0, T90 and T135) and urine collection in
902 metabolic cages for 5 days (T90, T135 and T180). No difference between GM versus non-GM diets
903 was observed. Similarly, gross necropsy findings did not provide evidence for a biologically relevant
904 difference between GM- versus non-GM feed, nor did macroscopic and evaluation of the organs
905 and tissues both in males and females. Microscopic histopathologic analysis identified few
906 abnormalities in animals fed the different diets, but a lack of evidence for a GM-diet effect. All these
907 results are in accordance to that reported previously by the GRACE EU-funded project (Zeljenková
908 *et al.*, 2014, 2016).

909 The main objective of the project was to identify biological and omics markers of exposure and
910 potentially of effects in order to discriminate a GM-based diet in comparison with a near isogenic
911 non-GM diet. The difference between GM- versus non-GM diets on hematologic parameters in
912 males only concerned the mean thrombocyte volume within the NK-G33 diet group, but not the
913 other groups, and at T90 but not at T180. Similarly, the difference in hematologic parameters in
914 females concerned the albumin/globulin ratio and eosinophils (%) in one diet group (respectively -
915 **NK11 and NK-G11) and at one feeding time (respectively T90 and T180). The difference between
916 GM- versus non-GM diet on clinical biochemistry parameters concerned triglycerides only in males
917 in the NK-G11 diet group and at T180. Kidney function is very often impaired earlier than other
918 functions in animals exposed to a wide variety of toxic agents and was previously reported altered
919 in rats fed with GM corn varieties by comparison with non-GM diet (de Vendômois *et al.*, 2009;
920 Séralini *et al.*, 2014). Consequently, we monitored urine parameters to assess kidney functions in
921 addition to necropsy and microscopic observations. No statistical difference was found in the effect
922 of GM- and non-GM diets. Despite few significant differences in biological parameters between the
923 diet groups, most of them correspond to a maize variety and environment effect and not to a GM

924 maize effect. We do not consider that these differences are biologically significant since none of
925 them showed a dose/response effect and numerous similar differences existed in other pairwise
926 comparisons. Our results are in accordance with the large majority of the reports (Bartholomaeus
927 *et al.*, 2013; Snell *et al.*, 2012) as well as with the GRACE EU-funded project (Zeljenková *et al.*,
928 2014, 2016; Schmidt *et al.*, 2017).

929 The investigations on reproductive function in males and females evidenced only marginal effects
930 in the male groups and none in the female groups as already reported for BT799, a maize
931 expressing the Cry1Ac gene (Guo *et al.*, 2015). Hormonal profiles in males and females were
932 established by immunological quantification in plasma and by the characterization of potential
933 disruptions induced by the diets at the steroidogenesis level analyzed by mass spectrometry
934 measurements of urine samples. We observed a difference in male urine samples collected after
935 90-day feeding only for pregnenolone and progesterone (**Table 5**). However, differences were
936 observed between the MON and NK-fed groups but not between MON or NK and their
937 corresponding non-GM controls. In addition, these differences were observed at T90 but not T180.
938 The biotransformation of pregnenolone into progesterone is mainly mediated by 3 β -hydroxy- δ 5-
939 steroid dehydrogenase (HSD3B) and the steroid δ -isomerase. The eventual inhibitory effect of the
940 diets on these enzymes would require additional investigation. In the case of a potential effect, the
941 inhibition mechanism will not rely on transcriptional regulation since no variation of the
942 corresponding mRNAs were found (supplementary Table 11).

943 The differences observed are not biologically relevant in light of the different GM percentages in
944 the diets, the sex, the experimental time, the links between the biological variables, the clinical
945 signs and the microscopic evaluation of the tissues. Most differences in biological parameters were
946 linked to the variety or environment effect in accordance with contaminants differentially recovered
947 in the MON versus NK-based diets. Then, the integration of data at T180 from 5 datasets (kidney
948 parameters, hormonal dosage, urine steroidome, biochemical data and organ weights) was
949 conducted per each sex. Despite the focus on diet discrimination, we could not obtain convincing
950 results to differentiate the diets.

951 The highly sensitive multi-omics approach was planned to decipher the complex physiological
952 response pattern of rats when exposed to diets despite the lack of substantial differences in
953 nutrient, anti-nutrients and contaminants between GM and non-GM as well as between NK and
954 NKG diets. Both mRNA and miRNA expression in liver and kidney differed between males and
955 females. However, the global analysis based on pairwise analyses of the diets showed a lack of
956 variation in miRNA between males and females. Some variations in mRNA expression between
957 the groups fed different diets were observed but no signature could be assigned to distinguish the
958 groups (**Fig. 4 and 5**). The pattern of DEGs between the groups and biochemical or pathological
959 parameters could not be assigned, as previously reported for rats fed with NK603 ± glyphosate
960 (Mesnage *et al.*, 2017). In fact, the pattern of mRNA expression between samples is currently used
961 to characterize sets of genes involved in specific metabolic pathways but not as a tool to
962 differentiate samples. In contrast, the metabolomic data discriminated the diets better when urine
963 was tested, rather than plasma. Possible explanations are that urine as the final metabolic
964 compartment of an organism cumulates effects, that metabolites are differentially present in the
965 two sample biofluids or that LC-HRMS (plasma) detects other types of metabolites than ¹H NMR
966 (urine). Indeed, since the composition of each diet was different, urine analysis could be expected
967 to discriminate the diets. Variations in metabolite concentrations were analyzed to investigate a
968 potential dietary effect linked to either GM food, glyphosate treatment or maize variety.

969 An effect of the GM-based diet was tested with both the MON or NK harvests. In contrast to the
970 effect observed by globally comparing the varieties (transcriptome and metabolome), only the data
971 from the urine metabolome allowed the diets to be differentiated. It is noteworthy that the chemical
972 analysis of urine also differentiated the eight types of pellets. The few significant differences
973 observed could be due to exposure to GM-based diets versus ISOMON or ISONK controls, but not
974 to a potential health effect. In fact, the search for an effect of GM-based food on various health
975 parameters such as kidney, liver and reproductive physiology was not fruitful, in accordance with
976 the findings of the GRACE project (Zeljenková *et al.*, 2014, 2016).

977 An effect of the treatment with glyphosate was tested although similar herbicide residue
978 concentrations (no significant difference) between diets were quantified. This did not allow us to
979 evidence any direct effect of glyphosate on rat health. In addition, owing to the low level of

980 contamination of the diets, glyphosate could not be quantified in kidney or liver extracts as
981 previously reported (Mesnage *et al.*, 2017). A few metabolites in urine, but not in plasma, allowed
982 the NKG- and NK-based diets to be differentiated. A slight decrease in taurine was found in females
983 with the NKG diets, with a dose effect apparent between the NKG33 and NKG11 diets at T135 and
984 T180. No such modification was found in males, but there was a slight increase in indoxylsulfate at
985 T90 and T135. Indoxylsulfate is a uremic toxin that is produced in the liver from indole, a tryptophan
986 derivative, of which increased levels are associated with chronic kidney disease (<3 μM in normal
987 conditions in humans vs >20 μM in uremia patients). However, neither histopathological nor
988 pathophysiologic examinations of kidneys indicated any damage. The slight variations in urine
989 metabolites cannot be associated with any biological disturbance, are not indicative of any health
990 effect and argue against an indirect effect of glyphosate. Other authors using transcriptomic and
991 metabolomic analyses of kidney and liver samples reached a similar conclusion (Mesnage *et al.*,
992 2017). Moreover, a recent transcriptomic analysis of gut tissues of rats fed with MON810 and the
993 near-isogenic control did not reveal any significant GM-related changes in expression profiles
994 (Sharbati *et al.*, 2017).

995 On the contrary, we could discriminate the groups fed with MON or NK varieties; this is in line with
996 the significant differences in the composition of the corresponding pellets attributed to the combined
997 effect of genotype and environment (Bernillon *et al.*, 2018). More specifically, the analysis of the
998 liver transcriptome in males allowed us partly to discriminate the two varieties (**Fig. 6**); a similar
999 result was obtained with the kidney transcriptome in males and females. Interestingly, common
1000 genes were expressed differently in both male livers and kidneys over time (T90 vs T180), such as
1001 Spw1 (selenoprotein W) and Gpx1 (glutathione peroxidase 1), which are both involved in oxidation-
1002 reduction reactions. Since fumonisins B1 and 2 alter the cellular redox balance (Rumora *et al.*,
1003 2007; Wang *et al.*, 2016), the increased expression of messengers coding for redox-sensitive
1004 signaling molecules (and potentially transcriptionally regulated by redox-sensitive transcription
1005 factors) might be due to the contamination of MON-based diets in comparison with NK-based diets
1006 (Chereau *et al.*, 2018). Fumonisins are mycotoxins produced by toxigenic *Fusarium* species
1007 characterized by a structural similarity to sphinganine and which consequently are able to strongly
1008 inhibit the ceramide synthase (Edite Bezerra da Rocha *et al.*, 2014). Despite a high number of

1009 DEGs at T180 in the kidney, no major signaling pathway was significantly identified in the whole
1010 sample. Metabolomic data on the grains showed that MON harvests had a higher content in
1011 betaine, proline, valine, alanine, GABA and succinic acid, whereas NK harvests had higher
1012 contents in several sugars or sugar alcohols, malic acid, fumaric acid, aspartic acid, glutamic acid,
1013 choline, tyrosine and tryptophan (Bernillon *et al.*, 2018). No clear correspondence between the
1014 metabolic data of the diets and plasma samples could be established. Several hypotheses could
1015 explain this finding: the composition of the pellets (only 33 % of maize), thereby minimizing
1016 differences between the maize harvests, the major role of the microbiota in the production of
1017 absorbed metabolites and slight differences in the nutritional composition of MON- and NK-based
1018 diets. In comparison, a clearer separation between the groups was observed using the
1019 metabolomics data obtained from urine. Based on PLS-DAs, the two varieties could be
1020 differentiated for both sexes, as shown in **Fig 7**. No link between maize kernel composition and the
1021 metabolic signature of exposure in urine could be established. In addition to variations in nutritional
1022 content, the two varieties were different in the nature and extent of their contaminants; as indicated
1023 above, a major difference was the 10-fold lower contamination of NK-based kernels by fumonisins
1024 than in MON kernels (Chereau *et al.*, 2018). The increased ratio of sphinganine/sphingosine in
1025 urine has been proposed as a biomarker of fumonisin exposure in humans (Solfrizzo *et al.*, 2004).
1026 However, the ¹H-NMR determination used in our study did not allow these metabolites to be
1027 quantified in urine.

1028 In conclusion, we identified no early biomarker of exposure or effect that could be added to the
1029 conventional 90-day rodent study required in the framework of the European regulation 503/2013
1030 and the multi-omics experiments did not bring new findings on a potential effect of GM-based diet.
1031 The results are in agreement with previous reports claiming the limited effect of GM feed in
1032 comparison with non-GM near-isogenic feed.

1033 At last, we showed in accordance with the results from GRACE and G-TwYST projects that the
1034 added scientific value of subchronic 90 day-studies animal feeding studies, without a targeted
1035 hypothesis might be limited and not significantly reduce remaining uncertainties (Bartholomaeus *et al.*
1036 *et al.*, 2013; Kuiper *et al.*, 2013). In the long term, 90-day or extended animal feeding studies might
1037 still be justified when there is a particular concern identified during the risk assessment procedures.

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Compliance with ethical standards:

The rodent experimentation conducted at CitoxLAB CRO was approved by the French Ethical Committee (CETEA) # 3148 -july 21, 2015.

Conflict of interest:

The authors declare no conflicts of interests. Public declaration of interests are available on the public RiskOGM programme website (<http://rechercheriskogm.fr/en/page/partners-pdis>).

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Tables

Table 1: Origins and composition of each diet (designated by a code, first column, the maize variety and content). All diets are composed of 33% of maize grain.

code	diet	maize variety	maize content
ACCLI*	conventional	SY NEPAL	33%
ISONK	closest near-isogenic NK603 non-GM maize	Pioneer 8906	33%
NK11	NK603 without glyphosate treatment (low dose)	Pioneer 8906R	11% NK603 + 22% ISONK
NK33	NK603 without glyphosate treatment (high dose)	Pioneer 8906R	33% NK603
NKG11	NK603 with glyphosate treatment (low dose)	Pioneer 8906R	11% NK603/glyphosate + 22% ISONK
NKG33	NK603 with glyphosate treatment (high dose)	Pioneer 8906R	33% NK603/glyphosate
ISOMON	closest near-isogenic MON810 non-GM maize	DKC6666	33%
MON11	MON810 (low dose)	DKC6667YG	11% MON810 + 22% ISOMON
MON33	MON810 (high dose)	DKC6667YG	33% MON810

* Conventional maize variety, from Koipesol Semillas

Table 2: Study plan. For each feeding condition, the composition of the diet is represented on the left part (dose) of the table. Each condition was subjected to the same experimental design (experimental time on the right part of the table) with three separate sub-groups (A, B, C).

DIET	DOSE (% W/W FEED)					T-14	EXPERIMENTAL TIME					rats per sex	
	control NK 603	NK 603	NK603 + glyphosate	control MON810	MON 810		T0	T90	T135	T180	sub-group		
ISONK	33					A c c l i m a t i o n	blood, urine, necropsy				A	10	
							blood	blood, urine	blood, urine	blood, urine, necropsy	B	12	
										blood, urine, necropsy	C	8	
NK11	22 11						blood, urine, necropsy				A	10	
							blood	blood, urine	blood, urine	blood, urine, necropsy	B	12	
										blood, urine, necropsy	C	8	
NK33	33						blood, urine, necropsy				A	10	
							blood	blood, urine	blood, urine	blood, urine, necropsy	B	12	
										blood, urine, necropsy	C	8	
NKG11	22 11		33			blood, urine, necropsy				A	10		
						blood	blood, urine	blood, urine	blood, urine, necropsy	B	12		
									blood, urine, necropsy	C	8		
NKG33	33					blood, urine, necropsy				A	10		
						blood	blood, urine	blood, urine	blood, urine, necropsy	B	12		
									blood, urine, necropsy	C	8		
ISOMON	33							blood, urine, necropsy				A	10
								blood	blood, urine	blood, urine	blood, urine, necropsy	B	12
											blood, urine, necropsy	C	8
MON11	22 11			33		blood, urine, necropsy				A	10		
						blood	blood, urine	blood, urine	blood, urine, necropsy	B	12		
									blood, urine, necropsy	C	8		
MON33	33					blood, urine, necropsy				A	10		
						blood	blood, urine	blood, urine	blood, urine, necropsy	B	12		
									blood, urine, necropsy	C	8		

Table 3a: Hematology of male samples at T180. Each tested parameter is represented by an individual line, each diet is represented by an individual column. In case of a statistically significant difference between two diets, a code (legend at the bottom of the table) is used to designate it in the last column.

Parameter	Diet								Difference between diets
	ISONK	NK11	NK33	NKG11	NKG33	ISOMON	MON11	MON33	
Red blood cells (10 ⁶ /μl)	n= 19 8.66 (0.52)	n= 17 8.74 (0.5)	n= 17 8.68 (0.45)	n= 18 8.61 (0.52)	n= 18 8.4 (0.6)	n= 19 8.78 (0.47)	n= 18 8.76 (0.54)	n= 20 8.76 (0.46)	
Haemoglobin (g/dl)	n= 19 14.79 (0.63)	n= 17 14.51 (0.53)	n= 17 14.72 (0.64)	n= 18 14.66 (0.76)	n= 18 14.33 (0.79)	n= 19 14.64 (0.6)	n= 18 14.46 (0.71)	n= 20 14.54 (0.5)	
Red differential weighin (%)	n= 19 13.34 (2.12)	n= 16 12.64 (1.47)	n= 17 12.78 (2.27)	n= 18 14.13 (3.55)	n= 18 13.02 (2.03)	n= 19 12.91 (1.7)	n= 18 13.69 (2.44)	n= 20 14.01 (4.53)	
Mean.corp.haem.conc. (g/dl)	n= 19 33.64 (0.77)	n= 17 33.17 (0.65)	n= 17 33.28 (0.53)	n= 18 33.39 (0.74)	n= 18 33.16 (0.77)	n= 19 33.04 (0.74)	n= 18 33.18 (0.79)	n= 20 32.98 (0.74)	12
Mean cell haemoglobin (pg)	n= 19 17.14 (1)	n= 17 16.62 (0.63)	n= 17 16.96 (0.62)	n= 18 17.06 (0.83)	n= 18 17.08 (0.88)	n= 19 16.69 (0.57)	n= 18 16.53 (0.81)	n= 20 16.64 (0.71)	
Reticulocytes (%)	n= 19 1.68 (0.29)	n= 17 1.62 (0.26)	n= 17 1.64 (0.3)	n= 18 1.53 (0.44)	n= 17 1.58 (0.32)	n= 19 1.59 (0.28)	n= 18 1.81 (0.56)	n= 20 1.55 (0.25)	
Mean cell volume (fl)	n= 19 50.94 (2.09)	n= 17 50.14 (1.53)	n= 17 50.98 (1.37)	n= 18 51.09 (1.81)	n= 18 51.52 (2.54)	n= 19 50.54 (1.72)	n= 18 49.81 (1.75)	n= 20 50.42 (1.4)	
Perox white blood cells (g/l)	n= 19 3.01 (0.86)	n= 17 2.71 (0.77)	n= 17 2.82 (0.65)	n= 18 2.84 (0.76)	n= 18 2.39 (0.72)	n= 19 2.8 (0.98)	n= 18 3.39 (1.19)	n= 20 2.54 (0.56)	3
Packed cell volume (l/l)	n= 19 0.44 (0.02)	n= 17 0.44 (0.02)	n= 17 0.44 (0.02)	n= 18 0.44 (0.02)	n= 18 0.43 (0.02)	n= 19 0.44 (0.02)	n= 18 0.43 (0.02)	n= 20 0.44 (0.02)	
Differential (g/dl)	n= 19 2.58 (0.29)	n= 17 2.49 (0.36)	n= 17 2.49 (0.32)	n= 18 2.58 (0.37)	n= 18 2.4 (0.22)	n= 19 2.6 (0.32)	n= 18 2.7 (0.31)	n= 20 2.58 (0.41)	
Platelets (10 ³ /μl)	n= 19 752 (83.63)	n= 17 757.06 (96.83)	n= 17 778.53 (76.61)	n= 18 769.83 (154.84)	n= 18 773.61 (148.53)	n= 19 760.63 (76.99)	n= 18 788.22 (102.72)	n= 20 729.2 (91.39)	
Mean thrombocyte volume (fl)	n= 19 6.93 (0.47)	n= 17 6.81 (0.39)	n= 17 6.9 (0.34)	n= 18 6.82 (0.47)	n= 18 7.09 (0.47)	n= 19 7.17 (0.48)	n= 18 7.12 (0.38)	n= 20 7.03 (0.43)	13
White blood cells (10 ³ /μl)	n= 19 2.82 (0.86)	n= 17 2.53 (0.73)	n= 17 2.69 (0.69)	n= 18 2.73 (0.75)	n= 18 2.24 (0.71)	n= 19 2.65 (0.94)	n= 18 3.18 (1.08)	n= 20 2.38 (0.52)	3, 13
Lymphocytes (10 ³ /μl)	n= 19 1.95 (0.72)	n= 17 1.87 (0.63)	n= 17 1.95 (0.69)	n= 18 1.94 (0.77)	n= 18 1.64 (0.66)	n= 19 2.02 (0.8)	n= 18 2.06 (0.65)	n= 20 1.77 (0.43)	
Lymphocytes (%)	n= 18 71.97 (6.07)	n= 17 73.14 (6.11)	n= 17 70.79 (14.28)	n= 18 74.47 (6.8)	n= 18 71.79 (10.36)	n= 19 75.51 (5.85)	n= 17 71.03 (7.21)	n= 20 73.71 (5.49)	
Neutrophils (%)	n= 18 22.9 (5.65)	n= 17 21.93 (5.45)	n= 17 24 (13.67)	n= 18 24.21 (14.53)	n= 18 23.24 (9.86)	n= 19 19.88 (5.13)	n= 17 24.17 (7.45)	n= 20 21.16 (5.26)	
Monocytes (%)	n= 18 1.79 (0.52)	n= 17 1.89 (0.86)	n= 17 2.08 (0.69)	n= 18 1.83 (0.67)	n= 18 2.04 (0.81)	n= 19 1.86 (0.67)	n= 18 1.96 (0.58)	n= 20 2.27 (0.46)	14
Eosinophils (%)	n= 18 2.36 (0.59)	n= 17 2.18 (0.57)	n= 17 2.18 (0.91)	n= 18 1.78 (0.43)	n= 18 2.09 (0.65)	n= 19 1.92 (0.56)	n= 18 1.96 (0.44)	n= 20 1.84 (0.55)	7, 10, 12
Basophils (%)	n= 19 0.05 (0.06)	n= 17 0.04 (0.05)	n= 17 0.03 (0.05)	n= 18 0.04 (0.06)	n= 18 0.04 (0.05)	n= 19 0.04 (0.06)	n= 18 0.05 (0.05)	n= 20 0.05 (0.06)	
Large unstained cells (%)	n= 19 0.95 (0.28)	n= 17 0.82 (0.42)	n= 17 0.94 (0.28)	n= 18 0.93 (0.43)	n= 18 0.8 (0.35)	n= 19 0.77 (0.26)	n= 18 0.88 (0.24)	n= 20 0.96 (0.32)	

Statistical difference between diets with the corresponding codes: 1: ISOMON versus MON11, 2: ISOMON versus MON33, 3: MON11 versus MON33, 4: ISONK versus NK11, 5: ISONK versus NK33, 6: NK11 versus NK33, 7: ISONK versus NKG11, 8: ISONK versus NKG33, 9: NKG11 versus NKG33, 10: NK11 versus NKG11, 11: NK33 versus NKG33, 12: ISOMON versus ISONK, 12: 13: MON11 versus NK11, 14: MON33 versus NK33.

The mean values per diet sub-group (sub-group B+C at T180) are reported with the standard deviation into brackets. The number of samples per diet is mentioned as (n).

Table 3b: Hematology of female samples at T180. Each tested parameter is represented by an individual line, each diet is represented by an individual column. In case of a statistically significant difference between two diets, a code (legend at the bottom of the table) is used to designate it in the last column.

Parameter	Diet								Difference between diets
	ISONK	NK11	NK33	NKG11	NKG33	ISOMON	MON11	MON33	
Red blood cells (10 ⁹ /μl)	n= 19 7.57 (0.33)	n= 16 7.33 (0.62)	n= 15 7.29 (0.31)	n= 19 7.52 (0.39)	n= 18 7.47 (0.38)	n= 18 7.51 (0.35)	n= 19 7.39 (0.4)	n= 16 7.55 (0.32)	14
Haemoglobin (g/dl)	n= 19 13.82 (0.56)	n= 16 13.66 (0.65)	n= 15 13.67 (0.52)	n= 19 13.86 (0.45)	n= 18 13.89 (0.68)	n= 18 13.69 (0.53)	n= 19 13.69 (0.58)	n= 16 13.66 (0.57)	
Red differential weighin (%)	n= 19 11.28 (1.59)	n= 15 12.41 (1.98)	n= 14 11.49 (1.25)	n= 19 12.28 (4.01)	n= 18 11.85 (1.88)	n= 18 11.47 (1.59)	n= 19 11.75 (1.57)	n= 16 11.78 (2.67)	
Mean.corp.haem.conc. (g/dl)	n= 19 33.8 (0.75)	n= 16 33.98 (0.89)	n= 15 34.53 (0.62)	n= 19 33.98 (0.97)	n= 18 34.27 (0.77)	n= 18 34.21 (0.77)	n= 19 34.3 (0.58)	n= 16 34 (0.67)	5, 14
Mean cell haemoglobin (pg)	n= 19 18.27 (0.63)	n= 16 18.76 (1.38)	n= 15 18.75 (0.71)	n= 19 18.48 (0.86)	n= 18 18.6 (0.61)	n= 18 18.23 (0.62)	n= 19 18.57 (0.5)	n= 16 18.12 (0.68)	14
Reticulocytes (%)	n= 19 1.94 (0.47)	n= 15 2.19 (0.71)	n= 15 1.96 (0.49)	n= 19 1.98 (0.57)	n= 18 2.05 (0.47)	n= 18 1.8 (0.5)	n= 19 2.24 (0.66)	n= 16 1.92 (0.53)	10
Mean cell volume (fl)	n= 19 54.05 (1.84)	n= 15 54.25 (1.99)	n= 15 54.28 (1.53)	n= 19 54.35 (1.67)	n= 18 54.27 (1.49)	n= 18 53.34 (1.12)	n= 19 54.16 (1.19)	n= 16 53.29 (1.42)	
Perox white blood cells (g/l)	n= 19 1.97 (1.31)	n= 16 2.07 (0.78)	n= 15 1.58 (0.41)	n= 19 1.64 (0.5)	n= 18 1.71 (0.65)	n= 18 1.59 (0.41)	n= 19 1.67 (0.46)	n= 16 1.61 (0.41)	
Packed cell volume (l/l)	n= 19 0.41 (0.02)	n= 16 0.4 (0.02)	n= 15 0.4 (0.02)	n= 19 0.41 (0.02)	n= 18 0.4 (0.02)	n= 18 0.4 (0.01)	n= 19 0.4 (0.02)	n= 16 0.4 (0.02)	
Differential (g/dl)	n= 19 2.02 (0.2)	n= 16 2.18 (0.19)	n= 15 2.17 (0.31)	n= 19 2.1 (0.28)	n= 18 2.14 (0.21)	n= 18 2.16 (0.22)	n= 19 2.19 (0.19)	n= 16 2.07 (0.23)	12
Platelets (10 ³ /μl)	n= 19 804.63 (117.96)	n= 16 853.5 (127.55)	n= 15 832.13 (105.21)	n= 19 801.11 (101.76)	n= 18 776.67 (94.12)	n= 18 796.33 (76.08)	n= 19 830.32 (126.9)	n= 16 795.31 (54.28)	
Mean thrombocyte volume (fl)	n= 19 7.13 (0.66)	n= 16 7.03 (0.67)	n= 15 6.76 (0.46)	n= 19 7.06 (0.55)	n= 18 7.11 (0.51)	n= 18 7.11 (0.39)	n= 19 7.11 (0.47)	n= 16 7.15 (0.38)	11, 14
White blood cells (10 ³ /μl)	n= 19 1.83 (1.16)	n= 16 1.97 (0.77)	n= 15 1.48 (0.39)	n= 19 1.54 (0.49)	n= 18 1.63 (0.68)	n= 18 1.46 (0.39)	n= 19 1.56 (0.4)	n= 16 1.52 (0.44)	13
Lymphocytes (10 ³ /μl)	n= 19 1.28 (0.91)	n= 16 1.42 (0.72)	n= 15 0.95 (0.24)	n= 19 1.08 (0.4)	n= 18 1.19 (0.55)	n= 18 1 (0.34)	n= 19 1.03 (0.33)	n= 16 1.07 (0.31)	13
Lymphocytes (%)	n= 18 70 (7.71)	n= 16 70.27 (9.89)	n= 14 69.69 (6.07)	n= 19 69.15 (7.41)	n= 18 72.31 (6.57)	n= 17 69.77 (4.05)	n= 18 67.69 (7.41)	n= 16 70.47 (3.91)	
Neutrophils (%)	n= 19 26.73 (11.9)	n= 16 25.01 (9.31)	n= 15 27.75 (14)	n= 19 25.32 (7.2)	n= 18 22.69 (6.3)	n= 18 26.78 (9.89)	n= 19 28.44 (12.03)	n= 16 23.67 (3.66)	
Monocytes (%)	n= 19 2.56 (1.1)	n= 16 2.04 (0.66)	n= 15 2.33 (0.57)	n= 19 2.29 (0.64)	n= 18 2.33 (0.6)	n= 18 2.64 (0.63)	n= 19 2.67 (1.1)	n= 16 2.61 (0.68)	10, 13
Eosinophils (%)	n= 19 2.47 (1.14)	n= 16 2.12 (0.79)	n= 15 3.21 (1.2)	n= 19 2.67 (0.74)	n= 18 2.03 (0.58)	n= 18 2.54 (1.24)	n= 19 3.02 (1.35)	n= 16 2.63 (0.92)	6, 10, 11, 13
Basophils (%)	n= 19 0.11 (0.18)	n= 16 0.06 (0.09)	n= 15 0.04 (0.06)	n= 19 0.07 (0.07)	n= 18 0.1 (0.23)	n= 18 0.07 (0.09)	n= 19 0.05 (0.1)	n= 16 0.08 (0.09)	
Large unstained cells (%)	n= 19 0.51 (0.42)	n= 16 0.55 (0.34)	n= 15 0.43 (0.34)	n= 19 0.48 (0.38)	n= 18 0.54 (0.38)	n= 18 0.59 (0.35)	n= 19 0.58 (0.48)	n= 16 0.56 (0.46)	

Statistical difference between diets with the corresponding codes: 1: ISOMON versus MON11, 2: ISOMON versus MON33, 3: MON11 versus MON33, 4: ISONK versus NK11, 5: ISONK versus NK33, 6: NK11 versus NK33, 7: ISONK versus NKG11, 8: ISONK versus NKG33, 9: NKG11 versus NKG33, 10: NK11 versus NKG11, 11: NK33 versus NKG33, 12: ISOMON versus ISONK, 12: 13: MON11 versus NK11, 14: MON33 versus NK33.

The mean values per diet sub-group (sub-group B+C at T180) are reported with the standard deviation into brackets. The number of samples per diet is mentioned as (n).

Table 4a: Clinical biochemistry of male samples at T180. Each tested parameter is represented by an individual line, each diet is represented by an individual column. In case of a statistically significant difference between two diets, a code (legend at the bottom of the table) is used to designate it in the last column.

Parameter	Diet								Difference between diets
	ISONK	NK11	NK33	NKG11	NKG33	ISOMON	MON11	MON33	
Na+ (mmol/L)	n= 20 142.84 (0.74)	n= 20 142.85 (1.22)	n= 20 142.69 (0.95)	n= 20 142.68 (0.75)	n= 20 142.59 (0.88)	n= 20 143.03 (0.84)	n= 20 142.65 (0.71)	n= 20 142.59 (0.96)	
K+ (mmol/L)	n= 20 3.69 (0.41)	n= 20 3.63 (0.22)	n= 20 3.63 (0.21)	n= 20 3.82 (0.33)	n= 20 3.72 (0.26)	n= 20 3.8 (0.68)	n= 20 3.64 (0.25)	n= 20 3.7 (0.18)	10
Cl- (mmol/L)	n= 20 104.39 (1.55)	n= 20 103.83 (1.05)	n= 20 104.47 (0.94)	n= 20 104.3 (1.42)	n= 20 103.83 (1.45)	n= 20 103.64 (1.69)	n= 20 103.97 (0.91)	n= 20 103.5 (1.24)	11, 14
Ca++ (mmol/L)	n= 20 2.53 (0.06)	n= 20 2.53 (0.05)	n= 20 2.52 (0.07)	n= 20 2.51 (0.06)	n= 20 2.52 (0.06)	n= 20 2.55 (0.11)	n= 20 2.55 (0.04)	n= 20 2.54 (0.06)	
PHOS (mmol/L)	n= 20 1.41 (0.21)	n= 20 1.41 (0.25)	n= 20 1.41 (0.15)	n= 20 1.36 (0.21)	n= 20 1.39 (0.24)	n= 20 1.52 (0.27)	n= 20 1.45 (0.24)	n= 20 1.46 (0.17)	12, 14
GLUC (mmol/L)	n= 20 11.23 (1.35)	n= 20 11.24 (1.31)	n= 20 11.05 (0.98)	n= 20 11.6 (1.23)	n= 20 11.39 (1.23)	n= 20 11.45 (1.81)	n= 20 11.16 (1.28)	n= 20 12.23 (1)	14
UREA (mmol/L)	n= 20 5.75 (0.78)	n= 20 5.61 (0.87)	n= 20 5.79 (0.8)	n= 20 5.64 (0.86)	n= 20 5.57 (0.76)	n= 20 5.96 (0.83)	n= 20 5.99 (1.1)	n= 20 6.24 (0.8)	14
CREAT (μmol/L)	n= 20 39.76 (3.3)	n= 20 38.3 (3.87)	n= 20 38.55 (3.1)	n= 20 37.57 (4.05)	n= 20 39.28 (3.16)	n= 20 38.28 (4.39)	n= 20 37.56 (4)	n= 20 38.37 (3.43)	
TOT.BIL (μmol/L)	n= 5 1.08 (0.08)	n= 4 1.14 (0.05)	n= 2 1.13 (0.01)	n= 1 1.11 ()	n= 2 1.1 (0.12)	n= 2 1.06 (0.03)	n= 2 1.27 (0.26)	n= 4 1.2 (0.27)	
PROT (g/L)	n= 19 59.99 (2.16)	n= 20 59.88 (2.42)	n= 20 59.16 (1.39)	n= 20 59.3 (2.46)	n= 20 59.44 (2.02)	n= 20 60.08 (3.07)	n= 20 61.37 (2.05)	n= 20 59.81 (1.82)	13
ALB (g/L)	n= 19 35.74 (1.24)	n= 19 35.74 (0.99)	n= 20 35.2 (1.2)	n= 20 35.25 (1.48)	n= 20 35.7 (1.17)	n= 20 35.65 (1.79)	n= 20 36.05 (1.5)	n= 20 35.65 (1.23)	
A/G ratio	n= 20 1.48 (0.08)	n= 20 1.46 (0.06)	n= 20 1.47 (0.08)	n= 20 1.47 (0.07)	n= 20 1.51 (0.06)	n= 20 1.46 (0.09)	n= 20 1.43 (0.11)	n= 20 1.48 (0.06)	
CHOL (mmol/L)	n= 20 2.05 (0.23)	n= 20 2.1 (0.33)	n= 20 2.02 (0.34)	n= 20 1.9 (0.3)	n= 20 2.11 (0.33)	n= 20 1.99 (0.28)	n= 20 2.19 (0.32)	n= 20 2.11 (0.36)	
TRIG (mmol/L)	n= 20 1 (0.34)	n= 20 1.14 (0.37)	n= 20 1.24 (0.28)	n= 20 1.15 (0.46)	n= 20 1.24 (0.56)	n= 20 1.04 (0.47)	n= 20 1.15 (0.43)	n= 19 1.14 (0.36)	8, 9, 11
ALP (U/L)	n= 20 204.65 (56.05)	n= 20 234.85 (45.27)	n= 20 229.2 (63.76)	n= 20 222.6 (69.13)	n= 20 206.8 (52.57)	n= 20 211.95 (59.39)	n= 20 210.6 (64.64)	n= 20 216.3 (78.11)	
ASAT (U/L)	n= 20 93.75 (77.14)	n= 20 82.1 (19.63)	n= 20 77.4 (16.17)	n= 20 76.6 (26.85)	n= 20 79 (23.59)	n= 20 75.15 (32.46)	n= 20 84.2 (25.25)	n= 20 74.25 (18.47)	
ALAT (U/L)	n= 20 60.95 (63.03)	n= 20 54.85 (23.46)	n= 20 44.25 (10.38)	n= 20 47.7 (12.52)	n= 20 49.55 (20.12)	n= 20 46.15 (17.67)	n= 20 52.8 (20.96)	n= 20 49.1 (16.72)	
GGT (U/L)	n= 12 0.25 (0.62)	n= 5 0.2 (0.45)	n= 8 0 (0)	n= 11 0.09 (0.3)	n= 10 0.1 (0.32)	n= 12 0.08 (0.29)	n= 9 0.11 (0.33)	n= 10 0.2 (0.42)	
BIL.AC (μmol/L)	n= 14 27.65 (7.3)	n= 15 28.92 (4.16)	n= 14 26.35 (4.62)	n= 12 30.03 (12.99)	n= 15 27.42 (5.45)	n= 17 30.45 (10.12)	n= 17 30.04 (6.04)	n= 15 28.43 (11.69)	10

Statistical difference between diets with the corresponding codes: 1: ISOMON versus MON11, 2: ISOMON versus MON33, 3: MON11 versus MON33, 4: ISONK versus NK11, 5: ISONK versus NK33, 6: NK11 versus NK33, 7: ISONK versus NKG11, 8: ISONK versus NKG33, 9: NKG11 versus NKG33, 10: NK11 versus NKG11, 11: NK33 versus NKG33, 12: ISOMON versus ISONK, 12: 13: MON11 versus NK11, 14: MON33 versus NK33.

The mean values per diet sub-group (sub-group B+C at T180) are reported with the standard deviation into brackets. The number of samples per diet is mentioned as (n).

Table 4b: Clinical biochemistry of female samples at T180. Each tested parameter is represented by an individual line, each diet is represented by an individual column. In case of a statistically significant difference between two diets, a code (legend at the bottom of the table) is used to designate it in the last column.

Parameter	Diet								Difference between diets
	ISONK	NK11	NK33	NKG11	NKG33	ISOMON	MON11	MON33	
Na+ (mmol/L)	n= 20 141.54 (1.12)	n= 20 142.72 (1.04)	n= 20 141.78 (0.72)	n= 20 141.88 (1.17)	n= 19 142.05 (0.96)	n= 20 142.4 (0.97)	n= 20 141.68 (0.9)	n= 20 141.88 (1.15)	4, 6, 10, 11, 12, 13
K+ (mmol/L)	n= 20 4.03 (1.43)	n= 20 3.43 (0.36)	n= 20 3.57 (0.73)	n= 20 3.61 (0.85)	n= 19 3.4 (0.58)	n= 20 3.31 (0.2)	n= 20 3.51 (0.87)	n= 20 3.4 (0.22)	12
Cl- (mmol/L)	n= 20 104.11 (1.5)	n= 20 104.86 (1.73)	n= 20 105.36 (1.41)	n= 20 104.78 (1.74)	n= 19 105.18 (1.52)	n= 20 104.93 (1.66)	n= 20 104.69 (1.4)	n= 20 105.13 (1.4)	5
Ca++ (mmol/L)	n= 20 2.62 (0.18)	n= 20 2.56 (0.1)	n= 20 2.57 (0.1)	n= 20 2.57 (0.1)	n= 19 2.55 (0.09)	n= 20 2.55 (0.05)	n= 20 2.54 (0.1)	n= 20 2.55 (0.1)	
PHOS (mmol/L)	n= 20 1.28 (0.34)	n= 20 1.1 (0.28)	n= 20 1.08 (0.31)	n= 20 1.1 (0.28)	n= 19 1.19 (0.25)	n= 20 1.14 (0.29)	n= 20 1.16 (0.27)	n= 20 1.13 (0.34)	12
GLUC (mmol/L)	n= 19 10.53 (1.72)	n= 20 10.29 (0.91)	n= 20 9.96 (0.91)	n= 20 10.55 (1.13)	n= 19 10.29 (1.11)	n= 20 10.48 (1.09)	n= 20 10.38 (0.77)	n= 20 10.14 (1.12)	
UREA (mmol/L)	n= 20 5.86 (1.33)	n= 20 5.01 (0.79)	n= 20 5.5 (1)	n= 20 5.34 (0.98)	n= 19 5.21 (0.75)	n= 20 4.92 (0.91)	n= 20 5.44 (1.07)	n= 20 5.13 (1.12)	4, 12, 14
CREAT (μmol/L)	n= 20 37.55 (3.81)	n= 20 35.63 (3.01)	n= 20 37.08 (3.08)	n= 20 36.43 (2.78)	n= 19 36.18 (3.13)	n= 20 33.3 (3.49)	n= 20 34.85 (2.99)	n= 20 34.36 (3.57)	12, 14
TOT.BIL (μmol/L)	n= 11 1.46 (0.39)	n= 13 1.38 (0.28)	n= 18 1.49 (0.36)	n= 13 1.55 (0.48)	n= 15 1.68 (0.75)	n= 15 1.62 (0.7)	n= 17 1.53 (0.43)	n= 13 1.34 (0.3)	
PROT (g/L)	n= 20 67.09 (3.63)	n= 20 66.64 (4.7)	n= 20 68.12 (2.82)	n= 20 67.19 (3.42)	n= 19 65.75 (3.48)	n= 20 67.5 (3.79)	n= 20 66.86 (3.12)	n= 20 66.92 (3.47)	11
ALB (g/L)	n= 20 41.7 (2.56)	n= 20 41.15 (2.54)	n= 20 42.6 (1.98)	n= 20 41.8 (2.5)	n= 19 40.63 (2.34)	n= 20 41.55 (1.64)	n= 20 41.5 (1.96)	n= 20 41.7 (2.25)	11, 12
A/G ratio	n= 20 1.65 (0.11)	n= 20 1.63 (0.17)	n= 20 1.68 (0.13)	n= 20 1.65 (0.11)	n= 19 1.62 (0.13)	n= 20 1.61 (0.13)	n= 20 1.64 (0.13)	n= 20 1.66 (0.09)	
CHOL (mmol/L)	n= 20 2.19 (0.39)	n= 20 2.08 (0.57)	n= 20 2.07 (0.37)	n= 20 2.08 (0.51)	n= 19 1.97 (0.53)	n= 20 2.04 (0.44)	n= 20 2.22 (0.49)	n= 20 2.19 (0.6)	
TRIG (mmol/L)	n= 20 0.8 (0.53)	n= 20 0.73 (0.4)	n= 20 0.67 (0.46)	n= 20 0.64 (0.42)	n= 19 0.61 (0.42)	n= 20 0.46 (0.19)	n= 20 0.58 (0.28)	n= 20 0.57 (0.28)	12
ALP (U/L)	n= 20 93.85 (35.62)	n= 20 97.3 (55.18)	n= 20 98.85 (33.88)	n= 20 115.5 (66.77)	n= 19 111.58 (43.51)	n= 20 116.15 (73.86)	n= 20 117.5 (52.1)	n= 20 95.75 (36.75)	13
ASAT (U/L)	n= 20 77.6 (28.81)	n= 20 91.8 (39.14)	n= 20 87.3 (27.97)	n= 20 100.4 (29.61)	n= 19 91.74 (33.67)	n= 20 86.95 (26.35)	n= 20 93.5 (41.89)	n= 20 91.85 (37.67)	
ALAT (U/L)	n= 20 44.95 (16.68)	n= 20 44.45 (15.27)	n= 20 41.8 (8.89)	n= 20 54.6 (16.73)	n= 19 45.26 (20.54)	n= 20 43.05 (15.03)	n= 20 56.55 (32.68)	n= 20 64.15 (39.31)	14
GGT (U/L)	n= 11 0.18 (0.4)	n= 10 0.1 (0.32)	n= 11 0.09 (0.3)	n= 13 0.31 (0.48)	n= 13 0.38 (0.51)	n= 14 0.21 (0.43)	n= 15 0.2 (0.41)	n= 11 0.09 (0.3)	
BIL.AC (μmol/L)	n= 18 50.99 (18.72)	n= 12 72.38 (55.2)	n= 19 56.67 (38.74)	n= 15 61.18 (44.61)	n= 15 70.37 (58.48)	n= 14 71.36 (47.23)	n= 18 76.56 (57.35)	n= 20 46.16 (28.11)	

Statistical difference between diets with the corresponding codes: 1: ISOMON versus MON11, 2: ISOMON versus MON33, 3: MON11 versus MON33, 4: ISONK versus NK11, 5: ISONK versus NK33, 6: NK11 versus NK33, 7: ISONK versus NKG11, 8: ISONK versus NKG33, 9: NKG11 versus NKG33, 10: NK11 versus NKG11, 11: NK33 versus NKG33, 12: ISOMON versus ISONK, 12: 13: MON11 versus NK11, 14: MON33 versus NK33.

The mean values per diet sub-group (sub-group B+C at T180) are reported with the standard deviation into brackets. The number of samples per diet is mentioned as (n).

Table 5: Analysis of 19 urinary steroid hormones (steroidome) by mass spectrometry. The table reports the results of the comparison between the different groups of rats (males, females, T90 and T180) fed with the considered formulations in terms of urinary steroid hormone levels, for each gender and each sampling collection time (non-parametric Kruskal-Wallis test). Significant p-values ($p < 0.05$) are appears in red color. nd: non-determined due to non-detected/ $<LOD$ values or detection rate $< 50\%$.

Steroid Hormone	Males T90	Females T90	Males T180	Females T180
Pregnenolone	<0.001	0.223	0.436	0.078
17 α -OH-pregnenolone	nd	0.777	nd	0.307
DHEA	nd	0.628	nd	0.024
5-androstene-3 β ,17 β -diol	nd	0.315	nd	0.02
Progesterone	0.003	0.045	0.833	0.176
17 α -OH-progesterone	nd	0.093	nd	0.041
Androstendione	0.536	0.084	0.845	0.338
17 β -testosterone	0.481	0.081	0.262	0.22
5 α -pregnane-17 α -ol-3,20-dione_(17 α -OH-dihydroprogesterone)	nd	0.548	nd	0.231
5 α -Androstanedione	0.439	0.809	0.04	0.163
5 α -dihydrotestosterone_(5 α -DHT)	nd	0.02	nd	0.129
5 α -pregnane-3 α -ol-20-one_(Allopregnanolone)	nd	0.254	nd	0.276
5 α -pregnane-3 α ,17-diol-20-one_(17 α -OH-Allopregnanolone)	nd	0.534	nd	0.463
Androsterone	0.055	0.213	0.127	0.258
Epiandrosterone	nd	0.85	0.169	0.673
5 α -Androstane-3 β ,17 α -diol	nd	0.414	nd	0.034
5 α -Androstane-3 β ,17 β -diol	nd	0.681	nd	0.783
17 β -estradiol	nd	0.112	nd	0.379
Estrone	nd	0.052	nd	0.235

Table 6: Number of statistically differentially expressed genes (mRNA) or miRNA between each relevant diet condition (based on pairwise comparison between diets) in the rat liver or kidney samples at T90 and T180

		LIVER								KIDNEY							
		mRNA				miRNA				mRNA				miRNA			
		Female		Male		Female		Male		Female		Male		Female		Male	
		T90	T180	T90	T180	T90	T180	T90	T180	T90	T180	T90	T180	T90	T180	T90	T180
Glyphosate Effect	NK11 vs NKG11	0	0	0	0	0	0	0	0	0	0	0	3658	0	0	0	0
	NK33 vs NKG33	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Variety and Environment Effect	ISOMON vs ISONK	5	4	5	16	0	0	0	0	0	1	1	6	0	0	0	0
	MON11 vs NK11	4	8	90	284	0	0	0	0	0	1	3	795	0	0	0	0
	MON33 vs NK33	3	5	4	7	0	0	0	0	1	0	6	4	0	0	0	0
GM effect	MON11 vs ISOMON	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	MON33 vs ISOMON	1	0	0	0	0	0	0	0	0	0	0	822	0	0	0	3
	MON33 vs MON11	0	0	0	0	0	0	0	0	0	0	0	563	0	0	0	0
	NK11 vs ISONK	0	0	0	11	0	0	0	0	1	0	0	0	0	0	0	0
	NK33 vs ISONK	0	0	0	0	0	0	0	0	1	0	2	1	0	0	0	0
	NK33 vs NK11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 7: Number of statistically differential metabolites between each relevant diet condition (based on pairwise comparison between diets) in rat plasma or urine metabolomic profiles at T90, T135 and T180.

		PLASMA						URINE					
		Female			Male			Female			Male		
		T90	T135	T180	T90	T135	T180	T90	T135	T180	T90	T135	T180
Glyphosate Effect	NK11 vs NKG11	0	0	0	0	0	0	2	1	3	3	0	4
	NK33 vs NKG33	0	0	0	0	0	0	2	5	4	4	5	1
Variety and Environment Effect	ISOMON vs ISONK	0	1	0	1	3	7	0	3	3	3	4	11
	MON11 vs NK11	0	7	2	3	0	6	20	17	17	11	2	11
	MON33 vs NK33	0	4	1	4	0	2	13	7	8	6	5	11
GM effect	MON11 vs ISOMON	0	0	0	0	0	0	9	2	13	2	2	0
	MON33 vs ISOMON	0	0	0	0	0	0	1	3	8	0	1	3
	MON33 vs MON11	0	0	0	0	0	0	7	4	3	3	4	2
	NK11 vs ISONK	0	0	0	0	0	0	14	8	3	6	1	4
	NK33 vs ISONK	0	0	0	0	0	0	8	2	4	0	1	1
	NK33 vs NK11	0	0	0	0	0	0	1	4	2	4	1	2

Figure legends

Fig. 1: Hormones (testosterone, luteinizing hormone, LH and inhibin B) quantification by immunological assays. Intra-testicular (a, b) or blood (c, d) testosterone concentrations were determined in male rats at T90 (a, c) and T180 (b, d). Circulating LH was determined in males at T90 (e) and T180 (f) and the ratios of circulating testosterone versus LH (T/LH) level were determined at T90 (g) and T180 (h). Circulating inhibin B in males was determined at T90 (i) and T180 (j). The assay was performed with 10 (sub-group A) or 12 (sub-group B) samples per diet group respectively at T90 and T180. Adjusted p-values from Kruskal Wallis test (post-hoc Dunn's adjustment) are indicated (*: $p < 0.05$). NS: not significant.

Fig. 2: Box-plots presenting urinary concentrations of hormone steroids (pregnenolone, progesterone and 5α -androstenedione) in male rats (sub-group B). Determination of pregnenolone (a) and progesterone (b) concentrations at T90 (12 samples per diet group). Determination of 5α -androstenedione (c) concentrations at T180 (11-12 samples per diet group). Adjusted p-values from Mann-Whitney tests (post-hoc Tukey adjustment) are indicated (***: $p \leq 0.001$; **: $0.001 < p \leq 0.01$; *: $0.01 < p \leq 0.05$).

Fig. 3: Box-plots presenting urinary concentrations of hormone steroids (progesterone, 5α -DHT, DHEA, 5-androstene- $3\beta, 17\beta$ -diol, 17α OH-progesterone and 5α -androstane- $3\beta, 17\alpha$ -diol) in female rats (sub-group B). Determination of progesterone (a) and 5α -dihydrotestosterone (b) at T90. Determination of DHEA (c), 5-androstene- $3\beta, 17\beta$ -diol (d), 17α -hydroxyprogesterone (e) and 5α -androstane- $3\beta, 17\alpha$ -diol dehydroepiandrosterone (f) at T180. The measurement was performed with 10-12 samples per diet group. Adjusted p-values from Mann-Whitney tests (post-hoc Tukey adjustment) are indicated (***: $p \leq 0.001$; **: $0.001 < p \leq 0.01$; *: $0.01 < p \leq 0.05$).

Fig. 4: Principal component analysis using data generated with liver mRNA of rats sacrificed at T180. Each diet is identified by a specific color and correspondence is shown in Table 2. Each female is identified with a triangle while each male is identified with a circle; a) the first two principal

components explaining variability are represented on x-axis (PC1) and y-axis (PC2). A clear separation between males (circles) and females (triangles) is observed (PC1: 61% explained variability). b) the second and third principal components explaining variability are represented on x-axis (PC3) and y-axis (PC4). No clustering effect of the different diets is observed.

Fig. 5: PLS-DA (Partial least squares-Discriminant Analysis) between the eight diets based on liver transcriptome variables from males (circles) at T180; variability is represented on the x-axis (PC1) and the y-axis (PC2) (Fig. 2a), x-axis (PC3) and y-axis (PC4) (Fig. 2b), x-axis (PC4) and y-axis (PC5) (Fig. 2c), x-axis (PC6) and y-axis (PC7) (Fig. 2d). No clear clustering was observed between the eight diets.

Fig. 6: PLS-DA (Partial least squares-Discriminant Analysis) between the eight diets based on liver transcriptome variables from males at T180. A clustering is observed on the x-axis with a clear separation between both NK603 (left) and MON810 (right) diets.

Fig. 7: PLS-DA between eight diets based on urine metabolome variables at T90 a, d), T135 (b, e) and T180 (c, f); (a, b, c) females and (d, e, f) male rats. A clustering is observed on the x-axis with a clear separation between both MON810 (left) and NK603 (right) diets.

Fig.1

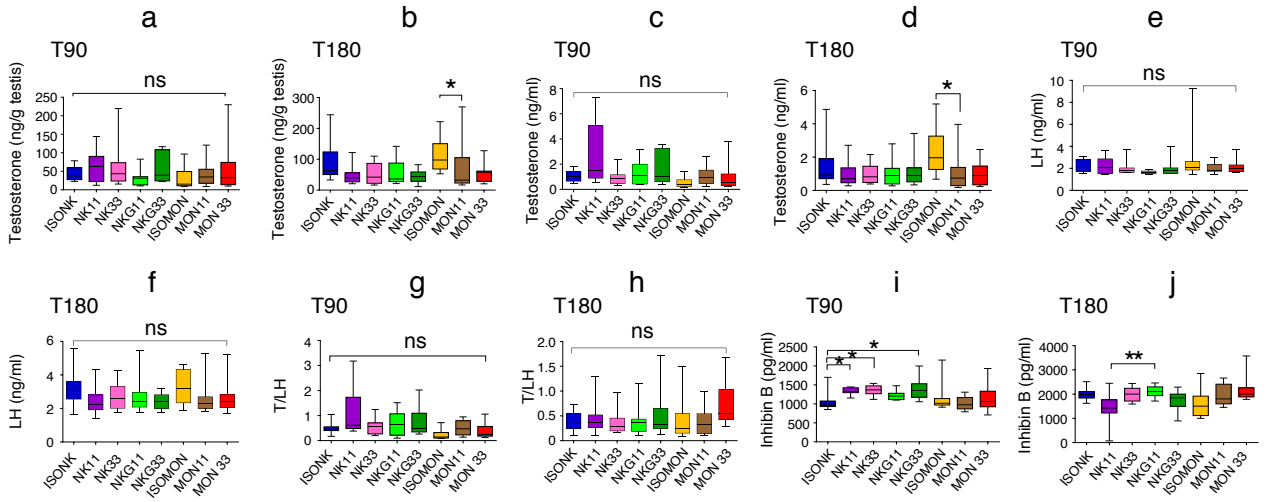


Fig. 2

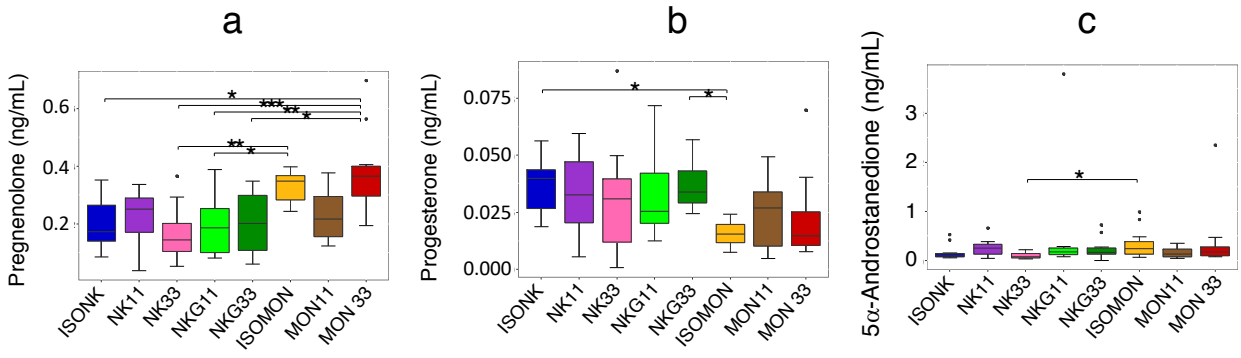


Fig. 3

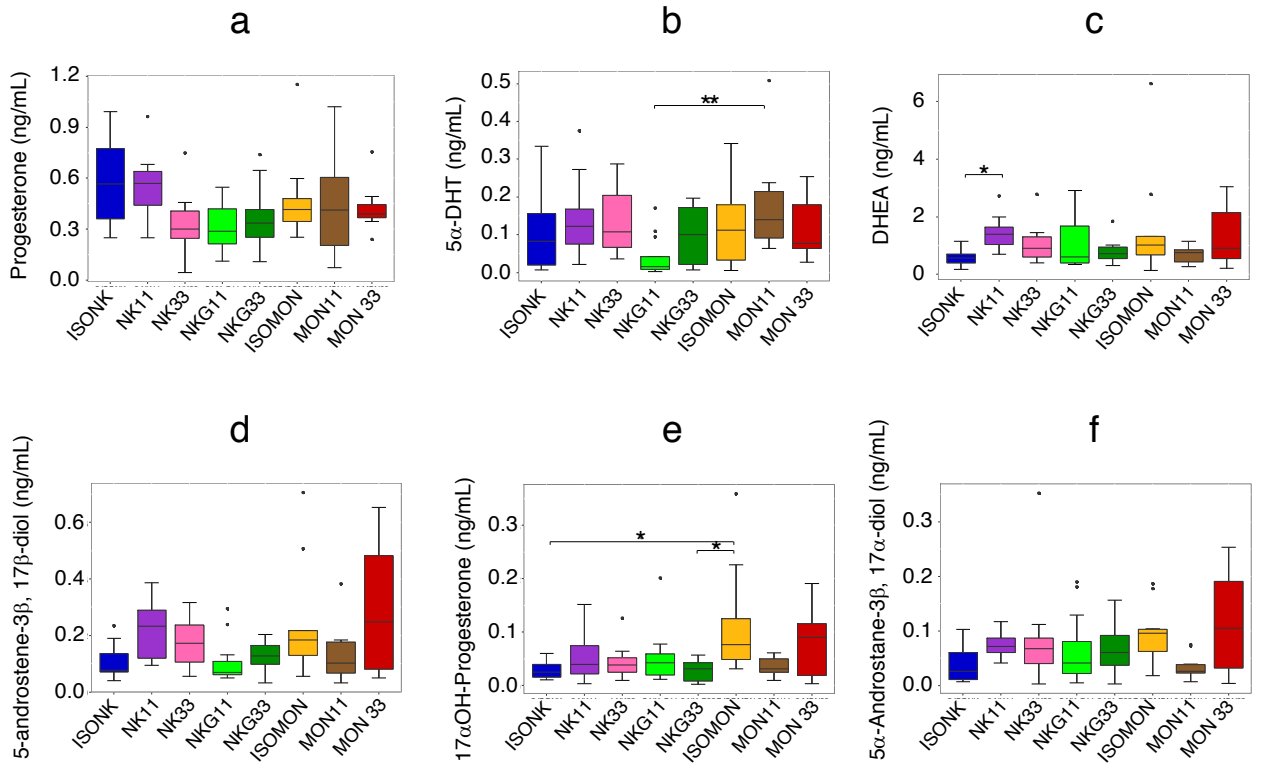


Fig. 4

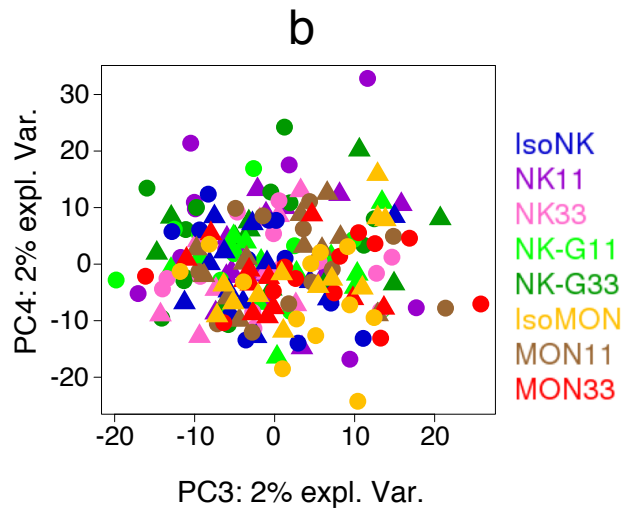
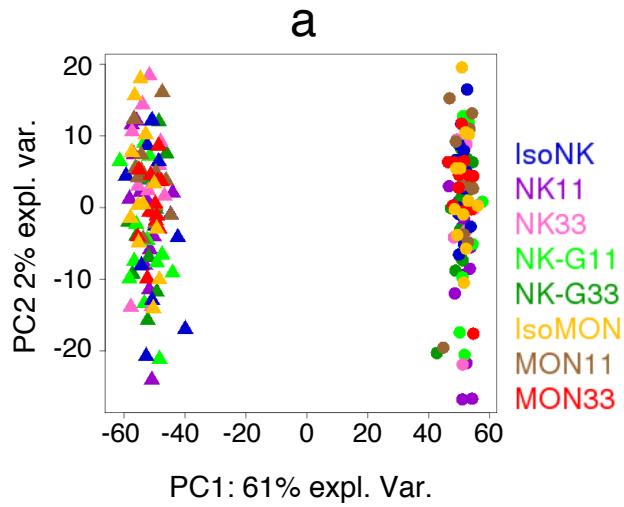


Fig. 5

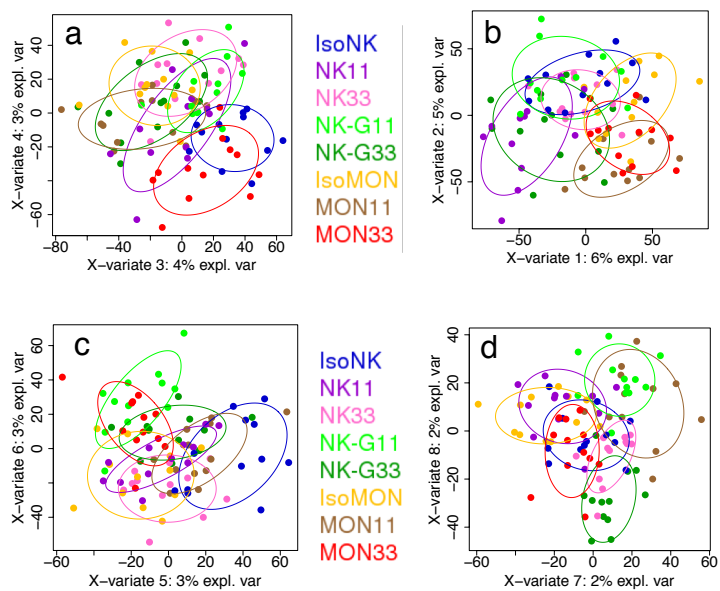


Fig. 6

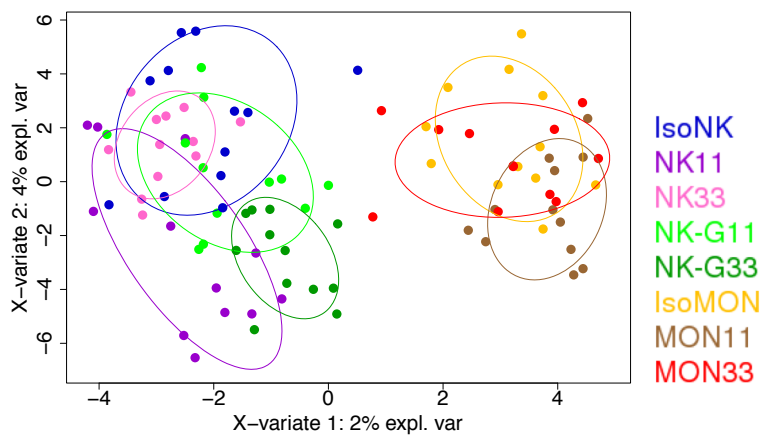


Fig. 7

