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Chronic exposure of adult, postnatal and *in utero* rat models to low-dose ^{137}Cesium: impact on circulating biomarkers

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ABSTRACT

The presence of ^{137}Cesium (^{137}Cs) in the environment after nuclear accidents at Chernobyl and more recently Fukushima Daiichi raises many health issues for the surrounding populations chronically exposed through the food chain. To mimic different exposure situations, we set up a male rat model of exposure by chronic ingestion of a ^{137}Cs concentration likely to be ingested daily by residents of contaminated areas (6500 Bq.l^{-1}) and tested contaminations lasting 9 months for adult, neonatal and fetal rats. We tested plasma and serum biochemistry to identify disturbances in general indicators (lipids, proteins, carbohydrates and electrolytes) and in biomarkers of thyroid, heart, brain, bone, kidney, liver and testis functions. Analysis of the general indicators showed increased levels of cholesterol (+26%), HDL cholesterol (+31%), phospholipids B (+15%) and phosphorus (+100%) in the postnatal group only. Thyroid, heart, brain, bone and kidney functions showed no blood changes in any model. The liver function evaluation showed changes in total bilirubin (+67%) and alkaline phosphatase (–11%) levels, but only for the rats exposed to ^{137}Cs intake in adulthood. Large changes in 17β-estradiol (–69%) and corticosterone (+36%) levels affected steroidogenesis, but only in the adult model. This study showed that response profiles differed according to age at exposure: lipid metabolism was most radiosensitive in the postnatal model, and steroid hormone metabolism was most radiosensitive in rats exposed in adulthood. There was no evidence of deleterious effects suggesting a potential impact on fertility or procreation.

KEYWORDS: ^{137}Cesium, chronic ingestion, low-dose, biomarkers

INTRODUCTION

The nuclear accidents at Chernobyl and more recently at Fukushima Daiichi caused a massive dispersal of radionuclides into the environment. Among those radionuclides common to both accidents are the noble gases, short-lived radionuclides (radioactive iodine and tellurides), and radioactive strontium and cesium. The longest-lived of the radionuclides detected in the environment after these accidents is ^{137}Cesium (^{137}Cs), with a half-life of 30.1 years. Its high solubility in water and high mobility in the environment results in its widespread distribution in plants and animals. The ^{137}Cs contamination from both accidents exceeds 600 000 Bq.m^{-2}, with ^{137}Cs deposits on 13 000 km^2 around the Chernobyl plant and on 600 km^2 around the Fukushima plant [1–3]. Thus, ^{137}Cs can concentrate in the food chain and contaminate surrounding territories in the long term.

Four years after Chernobyl, ^{137}Cs measurements in fresh food products showed contamination ranging from 25 to 200 Bq.kg^{-1}

[4]; 12 years later, the daily consumption of ^{137}Cs was ~100 Bq.kg^{-1}, without considering mushrooms, which were contaminated at concentrations reaching 2000 Bq.kg^{-1} [5]. The highest levels measured in food products near Fukushima were ~10–100 Bq.kg^{-1} only one year after the accident [1]. Thirty years after the Chernobyl accident, ^{137}Cs still remains in soil [6], and populations are still exposed to ^{137}Cs-contaminated foods [7]. ^{137}Cs is the prime source of long-term exposure to ionizing radiation; it is absorbed rapidly and completely and is distributed evenly throughout the body [8–10]. Exposure to ^{137}Cs raises many public health issues for people living in contaminated areas.

The epidemiological data collected since the Chernobyl accident show an increase in the frequency of thyroid cancer [11–13] and various types of leukemia [14, 15]—related mainly to iodine exposure and external irradiation, respectively. Epidemiological and medical studies about the thyroid status around Fukushima are currently under way, and so far they do not show any deleterious effects [16–18]. Studies also report higher than expected levels of non-cancer pathologies such as thyroid disorders in children [19], cardiovascular diseases in liquidators [20–22], renal disorders [23], brain damage [24–26], morphological bone defects in newborns [27], and sexual and physical developmental disorders related to hormone dysfunction in children [28]. Increased anxiety and stress have also been shown both around Chernobyl [29] and Fukushima [30], although without any dose–response relation to the contamination level of the respondents' homes. Overall, these studies demonstrate that there are undoubtedly health effects from radiation exposure in the post-accident situation. However, the relative roles of external radiation versus internal contamination, especially with ^{137}Cs, remain difficult to determine. ^{137}Cs might play a role in these post-accident non-cancer diseases. Previous results from our group have shown disturbances of the wakefulness–sleep cycle and of the cardiovascular system. Biological effects of ^{137}Cs on the metabolism of vitamin D, cholesterol, and steroid hormones were also described [31]. Moreover, the distribution of ^{137}Cs in rat tissues in our experimental model of chronic ingestion through drinking water was relatively homogeneous, with a global contamination of the organism [32].

To further characterize the consequences of chronic exposure to ^{137}Cs, we set up a food-chain model involving chronic ingestion of post-accident low-concentration ^{137}Cs. The ^{137}Cs concentration of 6500 Bq.l^{-1} used in this experiment is based on the maximum estimate for the dietary intake by those living around Chernobyl in the years after the accident [5]. We took age at exposure into account to assess the various sensitivities within the general population and thus improve prevention of health risks. Children are known to be more sensitive to pollutants [33, 34], and we can expect different physiological responses from them. Accordingly, we developed three models of exposure: an adult model, a postnatal model with a growing body, and an *in utero* model, in which the cascade of developmental processes is under way. For 9 months, rats were chronically exposed via their drinking water. Exposure was commenced for some rats during their adult life, for others immediately after birth, and for the remainder when they were embryos. The aim of this study was to investigate the effects of this exposure on general biochemical health markers in the blood and to assess the post-exposure functional integrity of major organs and tissues (thyroid, heart, brain, bone, kidney, liver and testis).

MATERIALS AND METHODS

Animals and ^{137}Cs administration

All experimental procedures were approved by the Animal Care Committee of the Institute of Radioprotection and Nuclear Safety and complied with French regulations for animal experimentation (*Ministry of Agriculture Act No. 87–848, 19 October 1987, modified 20 May 2001*).

Sprague–Dawley rats were obtained from Charles River Laboratories (L'Arbresle, France) for all exposure models. They were housed in pairs upon arrival and allowed to recover from transportation for 2 weeks before the experiment began. All rats were maintained in a 12-h light/12-h dark cycle (regular cycle) at 22 ± 1°C. Food and water were delivered *ad libitum*. The drinking water for the rats in the experimental group was contaminated with ^{137}Cs chloride ($^{137}CsCl_2$), obtained from CERCA (Pierrelatte, France), at a concentration of 6500 Bq.l^{-1}. Assuming that the daily consumption of an adult rat is between 25 and 30 ml of water, a concentration of 6500 Bq.l^{-1} of ^{137}Cs in water corresponds to an amount of 170 Bq/animal ingested per day. This daily intake is consistent with the estimations made for populations living in contaminated countries following the Chernobyl accident (between 20 and 2100 Bq.day^{-1}) [4, 5, 35] and is close to the intake of 100 Bq.day^{-1} (without considering mushrooms) measured for inhabitants living in the highly contaminated zone II of Christinovka (between 555 and 1480 kBq.m^{-2} of ^{137}Cs) [5]. Rats in the control group were given uncontaminated mineral water.

Adult, postnatal and *in utero* models (Fig. 1)

Adult contamination

The adult model used rats aged 3 months at the onset of the exposure to ^{137}Cs in their drinking water; they were 1 year old at the end of exposure.

Postnatal contamination

Pregnant rats obtained 2 weeks after mating were individually housed upon arrival and randomly assigned to control and exposed groups. Contamination began at birth until 9 months of age. Dams received ^{137}Cs-containing water from the birth of the pups until weaning, so that pups were exposed via the dam's milk. Thereafter, the pups were exposed directly to ^{137}Cs through their drinking water. In contaminated areas, breast milk contains ^{137}Cs, and it enters the newborn's bloodstream and body [36, 37]. The transfer factor of ^{137}Cs through breastfeeding in the contaminated area of Belarus is about 15% [38].

In utero contamination

In the *in utero* model, male and female rats aged 12 weeks were housed in pairs (male and female separated) upon arrival. For the experiment, rats were divided into two groups per sex: control and ^{137}Cs-exposed. A week after contamination began, male rats were mated with females for 48 h (one male and two females in each group were housed together in the same cage.). Females then received ^{137}Cs-containing water during pregnancy, until weaning. Weaned 3 weeks after birth, the male offspring were housed in pairs from different mothers (assigned by randomization) and directly exposed

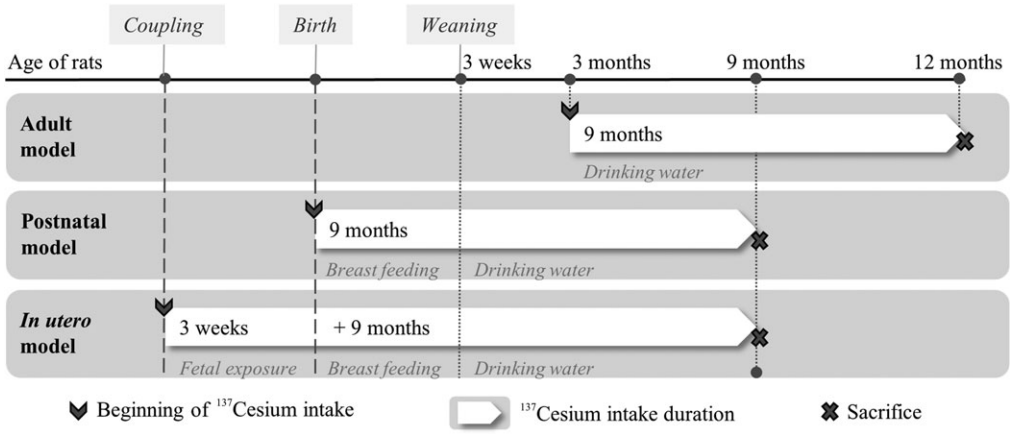

Fig. 1. Description of exposure models.

to ^{137}Cs-containing water until they were 9 months old. Female offspring and mothers were euthanized. It should be noted that the transfer of ^{137}Cs from mother to fetus is ~20% and that Cs accumulates in the fetus with increasing weight [39].

Sacrifice and biofluid collection

At the end of the exposure period, rats were anesthetized by inhalation of 5% isoflurane (Abbot France, Rungis, France) and euthanized by intracardiac puncture to collect blood. For plasma preparation, blood collected in heparinized tubes was centrifuged (1500 *g*) and supernatants were immediately frozen at −80°C. For serum preparation, we allowed the blood to clot by leaving it undisturbed at room temperature. After removing the clot by centrifuging (at 1500 *g*) for 10 min, the supernatant was frozen at −80°C.

^{137}Cs detection

After euthanasia, different organs (striated muscle, liver, kidney, brain, testicle and biofluids) were collected, weighed and stored dry to assess the contamination level (exposed versus control). ^{137}Cs was counted in these samples in a Cobra gamma counter with a NaI detector (Packard Instruments, Courtaboeuf, France) for at least 50 min per sample. Counts were made in the energy range of 620–720 keV, which corresponds to the main gamma ray energy of ^{137}Cs.

Blood biomarker assessment

Standard health biomarkers

Most biochemical indicators were measured in plasma samples with an automated spectrophotometric system (Konelab 20i from Thermo Fisher Scientific, Cergy-Pontoise, France), with the manufacturer's biological chemistry reagent (Brahms, Asnières sur Seine, France). The biomarkers measured were lipids (cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and phospholipids B), substrates (total protein, transferrin and glucose), electrolytes (calcium, phosphorus, magnesium, iron, chlorine, potassium and sodium), cardiac markers (creatine kinase (CK), the CKMB isoform of CK, and lactate dehydrogenase (LDH)), liver markers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, alkaline phosphatase, and albumin), and kidney markers (creatinine and urea). Phospholipid B (Diagnostic partners, Bormes Les Mimosas, France) and LDH (Diagam, Lille, France) indicators were adapted on the spectrophotometric system.

Thyroid markers

Plasma thyroid-stimulating hormone (TSH) was determined with the TSH rat ELISA kit from MP Biomedicals (Illkirch-Graffenstaden, France). Plasma free triiodothyronine (free T3) and free thyroxine (free T4) levels were determined by immunoassay on an IMMULITE® 2000 system from Siemens (Saint-Denis, France).

Brain marker

Plasma S100 Calcium Binding Protein B (S100B) was tested with an ELISA kit purchased from Euromedex (Souffelweyersheim, France).

Bone markers

Plasma 1,25(OH)2D3 (active Vitamin D) and 25(OH)D3 (the main circulating form) were assayed with a 1,25-Vitamin D EIA kit and a 25-hydroxyvitamin D RIA kit (Immunodiagnostic systems, Paris, France). Parathyroid hormone (PTH) was determined with the Rat Intact PTH ELISA Kit (Immunodiagnostic Systems, Paris, France).

Steroidogenesis hormones

For the adult model, serum hormone assays (testosterone, 17β-estradiol, corticosterone and aldosterone) were performed by ELISA kits from Abcys (Paris, France), in accordance with the manufacturer's instructions. For the postnatal and *in utero* models, plasma testosterone, 17β-estradiol (DSL, Cergy-Pontoise, France), follicle-stimulating hormone (FSH) (Amersham Pharmacia, Orsay, France) and luteinizing hormone (LH) (Biocode Hycel, Pouilly en Auxois, France) were measured by RIA, in accordance with the manufacturer's instructions.

Statistics

Results are expressed as means ± 95% confidence limit. Unpaired Student's *t*-tests were routinely performed for statistical analyses,

but were replaced by the Mann–Whitney rank sum test when the equal variance test failed (determined by SigmaStat software). Differences were considered statistically significant when $P < 0.05$ ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

RESULTS

^{137}Cs accumulation and dose estimates

^{137}Cs was measured in some selected organs to assess the level of contamination of the animals and its homogeneity in each group. Regardless of the contamination schedule, striated muscle was the site of the highest ^{137}Cs concentrations (~10 Bq.g^{-1}). The mean ^{137}Cs radioactivity measured in urine was slightly less than 10 Bq.g^{-1}. Concentrations measured in thyroid, kidney, liver, testis and heart samples ranged around 5 Bq.g^{-1}. ^{137}Cs radioactivity in the brain was ~2.5 Bq.g^{-1}, and in plasma it was below the limit of detection (between 1.53 Bq.g^{-1} of tissue and 4.43 Bq.g^{-1} of tissue, depending on the sample weight and counting time). Although the number of organs used for ^{137}Cs measurements were limited, the results were in agreement with previous biokinetics experiments using a similar exposure schedule [32]. Thus, based on previously published biokinetics data [32] and using dose conversion factors proposed by ICRP publication 108 [40, 41], the mean absorbed radiation dose was estimated at 4.4 ± 1.3 mGy for the adult model in these previous experiments. The absorbed radiation doses in the postnatal model and in the *in utero* models were slightly higher. In fact, the estimated absorbed radiation dose during the lactation period was 0.30 ± 0.05 mGy, based again on these previous experiments [32]. Regarding the *in utero* period, a previous study showed that 85% of the absorbed radiation dose was due to the ^{137}Cs burden of the mother [41]. The estimated absorbed radiation dose during the *in utero* period was thus 0.96 ± 0.15 mGy.

General health status

The food and water intake of each group was monitored on a weekly basis throughout the entire exposure period, as was body weight. Exposure was not associated with food or water intake changes or weight modifications (data not shown). Macroscopic examination of the testes, livers, kidneys, hearts, lungs, intestines and brains showed that all appeared normal (organ weight, volume, shape), with no notable differences from the control rats.

Long-term effects: 9 months of exposure of three rat models

Adult model

Table 1 shows that in the adult model, the plasma levels of numerous markers of homeostasis did not differ from those of controls after 9 months of ^{137}Cs exposure. Indeed, the only differences observed between the exposed and control groups involved only three types of functions, and then only some of the indicators. Hepatic function changed in the ^{137}Cs-exposed adult group, as measured by total bilirubin, which increased (+67%, $P < 0.01$), and alkaline phosphatase, which decreased (–11%, $P < 0.05$), but neither transaminase levels nor the AST/ALT ratio changed. For testicular steroidogenesis, the blood level of 17β-estradiol decreased (–69%, $P < 0.05$) in the exposure group (but testosterone did not change). Similarly, blood corticosterone levels, which assessed adrenal steroidogenesis, increased (+36%, $P < 0.05$) in exposed rats compared with control rats, while aldosterone levels did not change.

Postnatal model

As Table 2 shows, ^{137}Cs exposure affected some general homeostatic indicators in the postnatal model (Table 2). We observed disturbances in the lipid status, with levels of cholesterol (+26%, $P < 0.001$), HDL-cholesterol (+31%, $P < 0.01$) and phospholipids (+15%, $P < 0.01$) increasing in the exposed group. Plasma phosphorus was twice as high in the exposure group compared with that in the control group ($P < 0.001$). No thyroid hormones differed, nor did any heart, liver, kidney, brain or bone functions. Finally, no aspects of steroidogenesis differed between the groups.

In utero model

Table 3 shows that none of the indicators examined at the end of the study period in the rats exposed from early in the fetal period differed from those of the control rats.

DISCUSSION

The long-term effects of post-accident exposure to low-dose ^{137}Cs are still unclear, and discussions about the health consequences for exposed populations regularly take place, sometimes reporting contradictory results. This work assessed the effects on blood biomarkers of daily ingestion of ^{137}Cs in rats for 9 months beginning (i) in adulthood, (ii) at birth (including 3 weeks of breastfeeding from dams given ^{137}Cs-containing water) and (iii) in the fetal stage (from 3 weeks before birth until 9 months of age). For these conditions, no visible deleterious effects on general health indicators such as food or water intake or body weight were shown for any of these groups [42, 43].

The advantage of measuring markers in the blood to determine the results of exposure to low doses of ionizing radiation has previously been demonstrated experimentally [44, 45]. Accordingly, we evaluated blood biochemistry to identify possible disruptions in general indicators (lipids, proteins, carbohydrates and electrolytes], and biomarkers of thyroid, heart, liver, kidney, brain, bone and testis (steroidogenesis) impairment.

First, we measured lipids, proteins, carbohydrates and electrolytes in plasma, because they are essential to the maintenance of homeostasis and therefore of physiological functions. For instance, a lipid imbalance, such as elevated triglycerides or LDL cholesterol, may point to cardiovascular impairment [46]. Similarly, changes in calcium and phosphorus concentrations associated with modifications in PTH or 1,25(OH)2D3 plasma levels may indicate mineral and bone disorders or phosphorus diabetes [47]. Our results showed that ^{137}Cs exposure for 9 months beginning in adulthood or during gestation did not alter lipid, protein, carbohydrate or electrolyte levels in rats, although additional experiments for these two models of exposure showed some molecular-level and protein-level changes in cholesterol metabolism [43, 48, 49]. In the postnatal model, ^{137}Cs exposure increased levels of cholesterol, HDL cholesterol, phospholipids and phosphorus, although these changes were

Table 1. Biochemical indicators in plasma and serum for the adult model

Function		Blood biomarkers	Adult model	
			Control	^{137}Cs
General indicators:	Lipids	Cholesterol (mmol/l)	2.76 ± 0.36 (15)	2.96 ± 0.48 (15)
		HDL cholesterol (mmol/l)	1.73 ± 0.27 (15)	1.98 ± 0.29 (15)
		LDL cholesterol (mmol/l)	0.44 ± 0.15 (15)	0.62 ± 0.36 (15)
		Phospholipids B (g/l)	1.94 ± 0.24 (15)	2.13 ± 0.23 (15)
		Triglycerides (mmol/l)	2.06 ± 0.62 (15)	1.84 ± 0.42 (15)
	Other substrates	Total protein (g/l)	64.67 ± 2.20 (15)	64.26 ± 5.80 (15)
		Transferrin (g/l)	3.28 ± 0.22 (15)	3.36 ± 0.17 (15)
		Glucose (mmol/l)	10.03 ± 0.74 (15)	10.98 ± 0.91 (15)
	Electrolytes	Calcium (mmol/l)	2.65 ± 0.07 (15)	2.64 ± 0.19 (15)
		Phosphorus (mmol/l)	1.36 ± 0.12 (15)	1.34 ± 0.20 (15)
		Magnesium (mmol/l)	ND	ND
		Iron (μmol/l)	37.08 ± 2.49 (15)	35.12 ± 3.21 (15)
		Chlorine (mmol/l)	96.63 ± 4.17 (10)	99.30 ± 4.57 (14)
		Potassium (mmol/l)	4.61 ± 0.23 (10)	4.66 ± 0.23 (14)
		Sodium (mmol/l)	121.80 ± 5.53 (10)	123.21 ± 5.25 (14)
Thyroid		TSH (ng/ml)	ND	ND
		Free T3 (pmol/l)	ND	ND
		Free T4 (pmol/l)	ND	ND
		Free T3/free T4	ND	ND
Heart		CK (U/l)	155.7 ± 39.6 (13)	195.54 ± 66.94 (15)
		CKMB (U/l)	283.6 ± 61.1 (14)	277.06 ± 61.80 (14)
		LDH (U/l)	217.8 ± 64.8 (14)	228.57 ± 61.94 (15)
Brain		S100B (pg/ml)	34.44 ± 17.64 (5)	23.32 ± 8.98 (4)
Bone		1,25(OH)D3 (pmol/l)	17.2 ± 8.81983793 (8)	11.60 ± 2.74 (8)
		25(OH)D3 (nmol/l)	39.2 ± 4.703913563 (8)	35.60 ± 2.74 (8)
		PTH (pg/ml)	126.5 ± 46.05915364 (8)	143.80 ± 41.94 (8)
Kidney		Creatinine (μmol/l)	48.74 ± 1.67 (15)	49.96 ± 1.89 (15)
		Urea (mmol/l)	6.06 ± 0.42 (15)	5.90 ± 0.43 (14)
Liver		ALT (U/l)	34.24 ± 15.82 (14)	42.19 ± 9.65
		AST (U/l)	63.06 ± 11.16 (11)	59.53 ± 12.66 (13)
		AST/ALT	1.87 ± 0.39 (11)	1.52 ± 0.25 (11)
		Direct bilirubin (μmol/l)	ND	ND

Continued

Table 1. Continued

Function	Blood biomarkers	Adult model	
		Control	^{137}Cs
	Total bilirubin (µmol/l)	3.55 ± 0.38 (15)	5.94 ± 1.17** (13)
	Alkaline phosphatase (U/l)	146.66 ± 45.12 (14)	129.36 ± 19.58* (15)
	Albumin (g/l)	33.56 ± 1.78 (15)	33.68 ± 2.80 (15)
Testis (steroidogenesis)	Corticosterone (pg/ml)	108.08 ± 29.87 (6)	147.92 ± 22.54* (6)
	Aldosterone (pg/ml)	290.65 ± 55.37 (6)	366.04 ± 58.92 (6)
	Testosterone (ng/ml)	0.36 ± 0.06 (6)	0.57 ± 0.16 (6)
	17β-estradiol (pg/ml)	109.33 ± 51.74 (6)	33.61 ± 15.42* (6)
	LH (pg/ml)	ND	ND
	FSH (pg/ml)	ND	ND

Number of rats for each measurement is indicated in parentheses. Results are significantly different for: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ND = Not determined.

not associated with disruptions of the specific indicators of cardiac or bone function that we studied. These data suggest that in our experimental conditions, rats exposed from birth are more vulnerable to disorders of homeostasis than rats exposed in adulthood and *in utero*. This observation is rather unexpected, since fetal development is generally considered the stage most sensitive to exposures of any kind [50]. However, since there is only one time-point in our study, one can suppose that changes in these indicators may occur at an earlier time-point of exposure. This could suggest that adaptive mechanisms took place during exposure.

Two types of non-cancer thyroid pathologies have been studied in populations exposed to fallout from the Chernobyl accident: thyroid nodules [19] and autoimmune thyroiditis [51]. Studies show a link between ^{131}Iodine contamination and these diseases [52, 53], although the possible role of additional radionuclides (including ^{137}Cs) and external radiation in the induction of these diseases remains uncertain. To study thyroid function thoroughly, we measured TSH, which is considered the most sensitive marker for this purpose, together with the free T3/T4 ratio [54]. Our results showed no changes in thyroid function for any exposure group. It must, nonetheless, be noted that the measurements of these biomarkers are missing for the adult group. Our results were consistent with those obtained for a study of children whose exposures to ^{137}Cs after the accident at Chernobyl were measured. That study showed no differences in average serum free thyroid hormones or TSH at any ^{137}Cs contamination level [55].

Some impairments of the cardiovascular system, including ECG alterations and arterial hypertension, were observed in children [56] and in liquidators after Chernobyl [57, 58]. Previous experimental results also show a link between chronic ^{137}Cs intake and changes in cardiovascular physiology. After 3 months of ^{137}Cs exposure in adult rats, mean blood pressure decreased, the circadian rhythm disappeared, and plasma concentrations of CK and CKMB increased [59]. Thus, changes in these biomarkers are to be expected after a longer exposure of 9 months. Moreover, as an analogue of potassium, which transmits nerve impulses and muscle contraction, including in the heart, ^{137}Cs can compete with potassium for transport in potassium channels and thus block it [60]. To evaluate possible cardiac dysfunction, we measured some biomarkers of cardiac injury used in standard clinical health assessments: CK, which is determined in patients with chest pain [61]; CK-MB (isoform of CK expressed in the heart) and LDH, two complementary biomarkers. Our measurements showed no evidence of cardiac impairment after ^{137}Cs exposure for any model. These results are contradictory to findings observed in experimental studies, most likely because of differences in dose levels and/or time of exposure between these studies.

Previous experimental research has shown important effects on brain and bone functions after chronic exposure to the same concentration of ^{137}Cs. Changes have been demonstrated in the metabolism of some neurotransmitters [62], at the electrophysiological level [63], and in the neuroinflammatory response [64]. In this work, we evaluated a possible neurodegenerative effect by measuring circulating S100B, which is described as a suitable marker for the diagnostic or prognostic assessment of neurodegeneration [65]. The absence of changes in S100B levels in any of the three exposure models seems to suggest the absence of a neurodegenerative effect after exposure under our conditions. Moreover, disturbances in the plasma level of the active form of vitamin D3 have been observed after chronic (3 months') exposure of adults [66] and in a postnatal model of exposure from the day of birth to the end of the lactation period, associated with changes in plasma levels of calcium, phosphorus and osteocalcin [67]. Bone function was evaluated by measuring the active and circulating forms of vitamin D3 and parathyroid hormone (which enhances the release of calcium contained in bones) [63]. It did not differ from controls in either the adult or postnatal models. These measurements are, however, missing for the *in utero* group. The absence of modification of these

Table 2. Biochemical indicators in plasma and serum for the postnatal model

Function		Blood biomarkers	Postnatal model	
			Control	^{137}Cs
General indicators:	Lipids	Cholesterol (mmol/l)	2.65 ± 0.20 (40)	3.35 ± 0.36*** (38)
		HDL cholesterol (mmol/l)	1.90 ± 0.12 (40)	2.50 ± 0.31** (37)
		LDL cholesterol (mmol/l)	0.38 ± 0.06 (40)	0.54 ± 0.15 (38)
		Phospholipids B (g/l)	1.83 ± 0.14 (40)	2.11 ± 0.15** (37)
		Triglycerides (mmol/l)	1.65 ± 0.23 (40)	1.98 ± 0.27 (38)
	Other substrates	Total protein (g/l)	66.45 ± 1.40 (40)	68.66 ± 1.69 (38)
		Transferrin (g/l)	2.22 ± 0.31 (40)	2.17 ± 0.37 (35)
		Glucose (mmol/l)	10.99 ± 0.52 (40)	11.23 ± 0.55 (38)
	Electrolytes	Calcium (mmol/l)	2.75 ± 0.04 (40)	2.72 ± 0.07 (38)
		Phosphorus (mmol/l)	1.55 ± 0.14 (40)	3.14 ± 0.79*** (37)
		Magnesium (mmol/l)	0.69 ± 0.04 (40)	0.71 ± 0.05 (25)
		Iron (μmol/l)	32.66 ± 1.99 (40)	35.25 ± 2.14 (38)
		Chlorine (mmol/l)	94.14 ± 1.41 (40)	94.04 ± 1.53 (38)
		Potassium (mmol/l)	4.30 ± 0.11 (40)	4.37 ± 0.13 (38)
		Sodium (mmol/l)	120.02 ± 3.64 (40)	119.26 ± 3.51 (38)
Thyroid		TSH (ng/ml)	2.02 ± 0.35 (11)	1.97 ± 0.43 (12)
		Free T3 (pmol/l)	4.29 ± 0.63 (6)	3.51 ± 0.92 (5)
		Free T4 (pmol/l)	18.77 ± 0.53 (6)	18.30 ± 1.35 (5)
		Free T3/free T4	0.23 ± 0.04 (6)	0.19 ± 0.04 (5)
Heart		CK (U/l)	261.7 ± 52.5 (40)	261.6 ± 78.1 (35)
		CKMB (U/l)	445.9 ± 97.4 (40)	477.7 ± 161.1 (35)
		LDH (U/l)	482.1 ± 105.0 (40)	530.3 ± 201.4 (35)
Brain		S100B (pg/ml)	53.33 ± 14.90 (11)	43.06 ± 18.85 (8)
Bone		1,25(OH)D3 (pmol/l)	29.6 ± 10.00 (8)	27.9 ± 5.10 (8)
		25(OH)D3 (nmol/l)	38.9 ± 2.94 (8)	38.3 ± 3.92 (8)
		PTH (pg/ml)	199.6 ± 60.56 (8)	159.7 ± 35.87 (8)
Kidney		Creatinine (μmol/l)	53.18 ± 1.33 (40)	53.04 ± 2.15 (38)
		Urea (mmol/l)	5.68 ± 0.42 (40)	5.63 ± 0.32 (38)
Liver		ALT (U/l)	54.04 ± 12.43 (40)	44.53 ± 4.94 (38)
		AST (U/l)	102.06 ± 18.36 (40)	91.19 ± 12.02 (37)
		AST/ALT	2.03 ± 10.15 (40)	2.13 ± 10.17 (37)
		Direct bilirubin (μmol/l)	3.53 ± 0.35 (26)	3.97 ± 0.44 (25)

Continued

Table 2. Continued

Function	Blood biomarkers	Postnatal model	
		Control	^{137}Cs
	Total bilirubin (µmol/l)	4.62 ± 0.52 (40)	5.25 ± 0.74 (37)
	Alkaline phosphatase (U/l)	123.16 ± 17.74 (40)	122.53 ± 13.48 (38)
	Albumin (g/l)	34.26 ± 1.02 (40)	34.07 ± 1.34 (38)
Testis (steroidogenesis)	Corticosterone (pg/ml)	ND	ND
	Aldosterone (pg/ml)	ND	ND
	Testosterone (ng/ml)	1.55 ± 0.73 (10)	1.45 ± 0.39 (10)
	17β-estradiol (pg/ml)	2.08 ± 0.92 (6)	2.76 ± 0.80 (9)
	LH (pg/ml)	0.18 ± 0.06 (5)	0.27 ± 0.10 (8)
	FSH (pg/ml)	7.21 ± 0.92 (10)	6.48 ± 1.47 (10)

Number of rats for each measurement is indicated in parentheses. Results are significantly different for: ** $P < 0.01$; *** $P < 0.001$; ND = not determined.

biomarkers is consistent with plasma calcium levels, which are not changed regardless of the exposure model. The effects observed in the earlier, shorter studies mentioned above [66, 67] were no longer present after prolonged ^{137}Cs exposure lasting for 9 months. These findings may reflect an adaptive response to the maintenance of brain and bone metabolisms over time in the presence of chronic ^{137}Cs contamination.

We also assessed the functional integrity of the liver and kidney, the organs of detoxification. Creatinine and urea, both important indicators of kidney function, and AST and ALT, two important transaminase enzymes, the increase of which is a good indicator of liver damage, were measured in plasma. Additional markers such as bilirubin, alkaline phosphatase, and albumin also completed the liver function evaluation. In the adult model, hepatic but not renal function was modified: we noticed an increase in total bilirubin and a decrease in alkaline phosphatase, but they were not associated with transaminase disorders or changes in the AST/ALT ratio used in the differential diagnosis of liver diseases [68]. A previous study of adult rats exposed to ^{137}Cs for 3 months reported disturbances in cholesterol and bile acid metabolism, together with an increase in CYP27A1 activity in the liver [48]. We observed no disturbances in the postnatal and *in utero* models. These results suggest the robustness of the detoxification system and a possible regulation of these functions that creates only minor changes from continuous, prolonged exposure to low-dose ^{137}Cs.

Steroidogenesis, which is essential for the synthesis of steroid hormones [69], has been shown to be a process sensitive to various exposures [70–72]. In our experimental conditions, we found hormonal changes in rats exposed from adulthood, with increased levels of corticosterone and decreased levels of 17β-estradiol, despite the lack of preferential ^{137}Cs accumulation in testes [32]. In contrast, the growth models showed no change in the levels of hormones involved in steroidogenesis after ^{137}Cs exposure, although corticosterone and aldosterone measurements are missing. In addition to these observations of plasma levels, molecular modifications in steroidogenesis metabolism have been shown in adult, postnatal, and *in utero* models after chronic ^{137}Cs exposure for 9 months [42, 73]. These experimental results showing an adult sensitivity disagree with those from the many studies that have shown increased sensitivity in children compared with adults after low doses of radiation [74] or chemical exposures [33, 75]. These effects might be due to the establishment of protective mechanisms in rats exposed to ^{137}Cs during development. They probably adapted, unlike rats exposed from adulthood, when the physiological systems are already in place at the onset of exposure: in these adult rats, steroidogenesis is disrupted. It is now necessary to assess the impact of these hormonal changes on reproduction by completing this study with functional experiments, in particular related to disorders of fertility and experiments conducted in the female. In addition, functional disturbances may affect procreation. The reproductive system develops earlier in females than in males, and the study of females may help to confirm the sensitivity of the steroidogenesis process in response to ^{137}Cs exposure.

^{137}Cs measurements in selected organs showed that ^{137}Cs concentrations were in close agreement with previously published biokinetic data [32]. However, the number of ^{137}Cs measurements was limited since the experiments presented here were not designed for a biokinetic purpose but for the measurement of numerous biochemical parameters. Thus it is not possible to directly estimate the absorbed radiation dose from ^{137}Cs measurements in the present study. However, assuming that the ^{137}Cs concentrations measured in the present study were similar to previous biokinetic experiments [32, 40, 41], it was thus possible to propose absorbed dose estimates according to the exposure schedule. The estimated absorbed radiation dose was 4.4 mGy in the adult model, 4.7 mGy in the postnatal model and 5.7 mGy in the *in utero* model. Exposure through placenta transfer and through lactation may account for 30% increase in total absorbed dose in the *in utero* model and 7% increase in total absorbed dose in the postnatal model as compared

Table 3. Biochemical indicators in plasma and serum for the *in utero* model

Function		Blood biomarkers	*In utero* model	
			Control	^{137}Cs
General indicators:	Lipids	Cholesterol (mmol/l)	2.71 ± 0.38 (10)	2.66 ± 0.33 (10)
		HDL cholesterol (mmol/l)	2.23 ± 0.38 (10)	2.14 ± 0.22 (10)
		LDL cholesterol (mmol/l)	0.40 ± 0.10 (10)	0.36 ± 0.08 (10)
		Phospholipids B (g/l)	1.96 ± 0.17 (10)	2.02 ± 0.17 (10)
		Triglycerides (mmol/l)	1.48 ± 0.53 (10)	1.65 ± 0.32 (10)
	Other substrates	Total protein (g/l)	68.51 ± 3.27 (10)	70.20 ± 2.94 (10)
		Transferrin (g/l)	1.78 ± 0.11 (10)	1.67 ± 0.14 (10)
		Glucose (mmol/l)	11.52 ± 1.08 (10)	11.39 ± 0.89 (10)
	Electrolytes	Calcium (mmol/l)	2.72 ± 0.07 (10)	2.68 ± 0.07(10)
		Phosphorus (mmol/l)	1.28 ± 0.08 (10)	1.28 ± 0.11 (10)
		Magnesium (mmol/l)	0.73 ± 0.02 (10)	0.72 ± 0.02 (10)
		Iron (µmol/l)	36.18 ± 3.05 (10)	39.36 ± 3.62 (10)
		Chlorine (mmol/l)	95.84 ± 1.51 (10)	96.35 ± 0.50 (10)
		Potassium (mmol/l)	4.23 ± 0.22 (10)	4.39 ± 0.20 (10)
		Sodium (mmol/l)	ND	ND
Thyroid		TSH (ng/ml)	2.01 ± 0.61 (7)	1.66 ± 0.45 (9)
		Free T3 (pmol/l)	3.56 ± 1.00 (5)	3.71 ± 0.39 (6)
		Free T4 (pmol/l)	20.08 ± 1.29 (5)	18.78 ± 0.55 (6)
		Free T3/free T4	0.18 ± 0.06 (5)	0.20 ± 0.02 (6)
Heart		CK (U/l)	716.2 ± 355.8 (10)	599.4 ± 263.4 (10)
		CKMB (U/l)	1212.1 ± 639.9 (9)	1383.4 ± 594.8 (9)
		LDH (U/l)	1455.9 ± 614.0 (10)	1458.8 ± 661.7 (10)
Brain		S100B (pg/ml)	45.88 ± 22.30439014 (6)	73.21 ± 22.73558222 (8)
Bone		1,25(OH)D3 (pmol/l)	ND	ND
		25(OH)D3 (nmol/l)	ND	ND
		PTH (pg/ml)	ND	ND
Kidney		Creatinine (µmol/l)	49.63 ± 3.37 (10)	52.89 ± 4.04 (10)
		Urea (mmol/l)	6.01 ± 0.33 (10)	6.17 ± 0.33 (10)
Liver		ALT (U/l)	60.58 ± 18.90 (10)	49.27 ± 8.51 (10)
		AST (U/l)	156.37 ± 41.78 (10)	146.70 ± 30.64 (10)
		AST/ALT	2.76 ± 0.68 (10)	3.07 ± 0.68 (10)
		Direct bilirubin (µmol/l)	4.88 ± 0.54 (10)	4.65 ± 0.60 (10)

Continued

Table 3. Continued

Function	Blood biomarkers	*In utero* model	
		Control	^{137}Cs
	Total bilirubin (μmol/l)	5.19 ± 0.91 (10)	5.15 ± 0.78 (10)
	Alkaline phosphatase (U/l)	127.87 ± 28.88 (10)	152.84 ± 13.12 (10)
	Albumin (g/l)	32.33 ± 0.94 (10)	32.85 ± 1.07 (10)
Testis (steroidogenesis)	Corticosterone (pg/ml)	ND	ND
	Aldosterone (pg/ml)	ND	ND
	Testosterone (ng/ml)	0.66 ± 0.24 (10)	0.77 ± 0.27 (10)
	17β-estradiol (pg/ml)	1.84 ± 0.74 (8)	1.10 ± 0.74 (10)
	LH (pg/ml)	0.26 ± 0.06 (9)	0.42 ± 0.29 (10)
	FSH (pg/ml)	3.90 ± 0.61 (10)	4.46 ± 0.59 (10)

Number of rats for each measurement is indicated in parentheses. ND = not determined.

with the adult model. These dose estimates indicate that absorbed radiation doses due to chronic ingestion of ^{137}Cs were low, close to the natural level of exposure. Moreover, these absorbed radiation doses were in the range of estimated exposures for populations living on territories contaminated by the Chernobyl accident [76].

There is no clear-cut link between the absorbed radiation dose estimates and the observed biological effect. In fact, the highest absorbed dose is ascribed to the *in utero* model, in which the least biological effects were observed. Moreover, significant biological effects were observed in various metabolic pathways according to the exposure schedule. Surprisingly, the biological effects appeared to be highly dependent on the exposure schedule rather than dependent on the absorbed radiation dose. In line with this, the absorbed radiation doses remained very low and similar to one another according to the exposure schedule. This suggests that the *in utero* model showed a higher ability to adapt to the presence of internal contamination by ^{137}Cs at low concentrations than did the adult model. This might be linked to the presence of adaptive mechanisms during development that are less efficient during adulthood.

The biomarkers chosen for this study were mainly indicators conventionally used in clinical chemistry. It would be useful to supplement this analysis by assessing more predictive biomarkers than those used in our study. Other indicators already shown to demonstrate radiation-induced damage include flt3-ligand (a marker of bone marrow involvement), citrullin (a marker of intestinal mucosa damage) and oxysterols (markers of tissue or metabolic disorders) [77]. Still other biomarkers appear promising, such as microRNAs, which have a high degree of specificity and sensitivity in differentiating pathologies and the ability to be detected rapidly and accurately [78]. Other non-targeted approaches could also provide some answers by allowing the identification of new biomarkers sensitive to exposures. For instance, metabolomic approaches for addressing the biological effects of contamination in animal models can discriminate ^{137}Cs-contamination from control animals, at chronic low doses [79] and at higher doses [80].

This study showed a response profile to ^{137}Cs that differed according to exposure model, and showed lipid metabolism disruption only in the postnatal model, and the greatest radiosensitivity for steroid hormone metabolism in animals exposed from adulthood. Changes in these biomarkers may be predictive of potential deleterious effects and should therefore undergo further study to help evaluate populations chronically exposed to low doses of ^{137}Cs. More generally, the assessment of plasma biomarkers in epidemiological studies could improve our understanding of the health effects attributed to nuclear accidents. It would now be interesting to develop this non-invasive biochemical approach by analyzing innovative circulating biomarkers.

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