Science of the Total Environment 542 (2016) 653–664

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluation of serum markers of blood redox homeostasis and inflammation in PCB naturally contaminated heifers undergoing decontamination

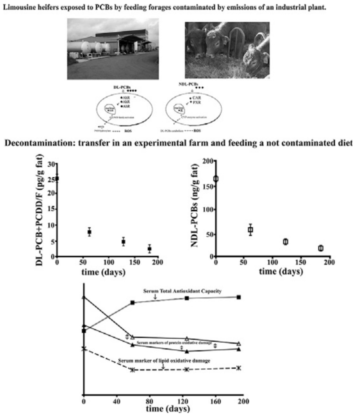
Luisa Cigliano [a], Carlo Nebbia [b], Guido Rychen [c], Cyril Feidt [c], Flavia Girolami [b], Cristina Rossetti [d], Maria Stefania Spagnuolo [d,]*

[a] *Department of Biology, University of Naples Federico II, via Cinthia 121, 80126 Naples, Italy*
[b] *Department of Veterinary Sciences, University of Turin, Largo P. Braccini 2, 10095 Grugliasco, Italy*
[c] *Université de Lorraine, INRA, 2 avenue de la forêt de Haye, TSA 40602, 54518 Vandoeuvre Cedex, France*
[d] *National Research Council (CNR), Institute of Animal Production System in Mediterranean Environments (ISPAAM), via Argine 1085, 80147 Naples, Italy*

HIGHLIGHTS

- Redox and inflammation indices were evaluated in heifers under decontamination.
- TEQ values of DL-PCBs + PCDD/Fs and NDL-PCBs content in pericaudal fat was measured.
- PCBs exposure negatively affects redox and inflammatory status of heifers.
- N-Tyr and TNF-alpha levels represent biomonitoring markers of decontamination.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 15 October 2015
Accepted 21 October 2015
Available online xxxx

Editor: Adrian Covaci

ABSTRACT

Dioxins and polychlorinated biphenyls (PCBs) are widely spread and long persistent contaminants. The aim of this study was to evaluate physiological changes associated with the decontamination of animals previously exposed to environmental pollutants. Eight Limousine heifers were removed from a polluted area and fed a standard ration for six months. The extent of contamination was defined by measuring total toxic equivalents (TEQ) values of dioxin like-PCBs (DL-PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), and NDL-PCBs amount in pericaudal fat two weeks after the removal from the contaminated area (day 0) and then bimonthly for six months during the decontamination (days 59, 125, and 188). The concentrations of both DL-PCBs + PCDD/Fs and NDL-PCBs at the start of decontamination (day 0) were higher than those legally admitted, and they were strongly decreased at the end of the experimental period. Specific

---

Abbreviations: Ret, Retinol; Toc, alpha-Tocopherol; Asc, ascorbic acid; N-Tyr, nitro-tyrosine; PCs, protein-bound carbonyls; LPOs, lipid hydroperoxides; SOD, superoxide dismutase; GPx, glutathione peroxidase; Hpt, Haptoglobin; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; DL-PCBs, dioxin-like polychlorinated biphenyls; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQs, toxic equivalents.

* Corresponding author at: Institute of Animal Production System in Mediterranean Environments (ISPAAM), via Argine 1085, 80147 Naples, Italy.
E-mail addresses: luisa.cigliano@unina.it (L. Cigliano), carlo.nebbia@unito.it (C. Nebbia), guido.rychen@univ-lorraine.fr (G. Rychen), cyril.feidt@univ-lorraine.fr (C. Feidt), flavia.girolami@unito.it (F. Girolami), cristina.rossetti@cnr.it (C. Rossetti), mariastefania.spagnuolo@cnr.it (M.S. Spagnuolo).

http://dx.doi.org/10.1016/j.scitotenv.2015.10.104

Keywords:
Biomarkers
Environmental pollution
PCB decontamination
Redox homeostasis
Haptoglobin
Oxidative stress

indices of blood redox homeostasis and inflammation were also measured at each time. Serum concentrations of Retinol, Tocopherol and Ascorbate, the total antioxidant capacity (TAC) and the activities of superoxide dismutase and glutathione peroxidase were lower at day 0 than after 59, 125 or 188 days of decontamination. Protein-bound carbonyls (PC), nitro-tyrosine (N-Tyr), and lipid hydroperoxides concentrations were higher at day 0 than during decontamination. In addition, TAC, PC and N-Tyr levels correlated with both DL-PCB and NDL-PCB concentrations only at day 0. Serum concentrations of TNF-alpha and Haptoglobin were higher in samples collected at day 0 than in those obtained during decontamination. As Haptoglobin and TNF-alpha levels correlated with both DL-PCB and NDL-PCB concentrations at day 0 and at day 59 (when these concentrations are still over legal limit), they might represent easily measurable parameters for assessing acute exposure to pollutants. Further both N-Tyr and TNF-alpha concentrations could be used as bio-monitoring markers of the decontamination procedure.

1. Introduction

Persistent organic pollutants (POPs) are toxic to human health and ecosystems, and are largely transferred in the food chain (Rychen et al., 2005; Antignac et al., 2006). Several POPs, such as dioxin-like and non dioxin-like polychlorinated biphenyls (DL-PCBs and NDL-PCBs respectively) are voluntary produced, for commercial purposes, as a result of industrial activities, while other pollutants, such as polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), designed as *dioxins*, are unintentional by-products of industrial processes. Dioxins and PCBs are toxic chemical compounds of great public health concern, and represent the major sources of environmental contamination. Due to their structural stability and volatility are widely spread, thus affecting agricultural areas near or far away from emitting sources (Beyer et al., 2000; Lohman and Seigneur, 2001). They are also resistant towards chemical and biological degradation processes, thus showing a long persistence in the environment. Although the Stockholm Convention on Persistent Organic Pollutants in 2001 banned PCB production internationally (UNEP, 2001), PCBs are still found in soil, fresh water, aquatic wildlife, and mammals, essentially because of the seepage into the environment, accidental spills, and improper disposal. Food chain contamination occurs as a consequence of the animal ingestion of contaminated water, soil and forage (McLachlan, 1993; Thomas et al., 1999a, 2002; Brambilla et al., 2004; Rychen et al., 2014). Indeed, dioxins and PCBs are lipophilic and accumulate in animal body, mainly in the liver and in the adipose fraction of organs and tissues (Larsen, 2006), being also transferred into milk and eggs. Therefore the consumption of animal products rich in fat represents by far the major source of exposure for humans (Thomas et al., 1999b; Schecter et al., 2006). However, ruminants were reported to be decontaminated (McLachlan, 1994; Thomas et al., 1999b; Huwe and Smith, 2005; Rossi et al., 2010; Rychen et al., 2014), essentially via milk excretion (Thomas et al., 1999b; Glynn et al., 2009; Rossi et al., 2010), or through the allometric increased volume of adipose tissue during growth (Chamberland et al., 1994; Glynn et al., 2009; Rychen et al., 2014).

Dioxins and DL-PCBs share a common toxicity mechanism, that is mediated via binding to a specific intracellular receptor, the aryl hydrocarbon receptor (AhR) (Alsharif et al., 1994; Mandal, 2005), whose activation is responsible for the enhanced expression of genes coding for cytochrome P450 1 family enzymes in liver of several species, including cattle (Safe, 1986; Whitlock, 1990; Machala et al., 1998; Matsumura, 2003; Guruge et al., 2009).

In particular, PCB 126, the most potent AhR agonist among DL-PCBs (Bandiera et al., 1982), not only alters the expression of genes coding for CYP1A1, but also impairs the expression of genes coding for antioxidant enzymes, resulting in oxidative stress in the liver (Hassoun et al., 2002; Parkinson et al., 1983). Dioxin exposure was reported to promote, via AhR activation, highly reactive oxygen species (ROS) production (Slezak et al., 2000; Nebert et al., 2000; Dalton et al., 2002), and to depress several ROS quenching systems (Ishida et al., 2009), thus inducing increased DNA fragmentation, as well as production of superoxide anion, thiobarbituric acid reactive substances, and hydroperoxides (Shertzer et al., 1995; Zhao and Ramos, 1998; Slezak et al., 2000, 2002). This leads to oxidative conditions (Shertzer et al., 1998; Slezak et al., 2000; Senft et al., 2002) that may induce oxidative stress-related processes (Mandal, 2005; Pelclova et al., 2011), that are associated with modifications of physiological and metabolic functions (Halliwell and Gutteridge, 2000). NDL-PCBs, due to their chemical properties, have a low affinity for AhR, and are mainly involved in alterations of signal transduction systems, neurotoxicity, immune suppression and endocrine disruption (Fischer et al., 1998; Selgrade, 2007). NDL-PCB bioaccumulate preferentially in adipose tissue causing disruption of lipid metabolism and induction of IL-6 and TNF-alpha production (Ferrante et al., 2015). NDL PCBs were also reported to reduce cell viability and induce oxidative stress (Westerink, 2014; Abella et al., 2015).

In physiological conditions, the antioxidant defence system, provided by enzymes and antioxidants, scavenges ROS, thus limiting or preventing oxidative damage (Halliwell and Gutteridge, 2000). Oxidative stress occurs as consequence of an imbalance between ROS production and neutralizing capacity of antioxidant mechanisms (Halliwell and Gutteridge, 2000), and is involved in the aetiology of several diseases and metabolic disorders (Lomba, 1996; Bernabucci et al., 2002, 2005; Castillo et al., 2005; Wilde, 2006), also contributing to the reduction of fertility in dairy cows (Wathes et al., 2012). Therefore, the evaluation of blood redox homeostasis has increasingly contributed to knowledge of the processes involved in reproductive and metabolic disorders (Campbell and Miller, 1998; Kankofer, 2002; Sordillo and Aitken, 2009), and it has become important as a complementary tool for the evaluation of health and metabolic status of dairy cows (Bernabucci et al., 2005; Castillo et al., 2005, 2006).

The main objective of this investigation was to evaluate the effect of a decontamination procedure, based on the removal of Limousine heifers from an agricultural area contaminated by emissions of an industrial plant specialized in PCB treatment, followed by the feeding a standard ration, on specific indices of blood redox homeostasis and inflammation, in order to assess the changes associated with decontamination, and to obtain tools for monitoring such a process. We focused on the analysis of oxidative and inflammatory status essentially because both DL-PCBs and NDL-PCBs, although through different pathways, are able to disrupt redox homeostasis and to induce inflammatory response.

2. Materials and methods

2.1. Materials

Bovine serum albumin fraction V (BSA), chemicals of the highest purity, Rabbit anti-human Hpt IgG, Goat anti Rabbit IgG-horseradish peroxidase linked (GAR-HRP), Rabbit anti-dinitrophenylhydrazine (anti-DNP) IgGs, and standards for high performance liquid chromatography (HPLC) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The Nucleosil 100-NH_2 column (5 μm particle size, 250 × 4.6 mm i.d.) and the Nova-PAK C18 column (4 μm particle size, 125 × 2 mm i.d.) were obtained from Macherey-Nagel (Duren, Germany). Organic solvents were purchased from Romil (Cambridge, UK). Polystyrene 96-wells plates were purchased from Nunc (Roskilde, Denmark). Nitrated BSA and the kit for titration of lipoperoxide of Cayman Chemical, as well as rabbit anti-nitrotyrosine IgG of Covalab were purchased by Vincibiochem (Vinci, Italy). The dye reagent for protein titration, recombinant bovine TNF-alpha, mouse anti-bovine TNF-alpha IgG, biotinylated anti-TNF-alpha IgG, and Streptavidin conjugated to HRP were from AbD Serotec (Bio-Rad Laboratories, Hercules, CA).

2.2. Farm selection and animals

The study was carried out on eight one year old contaminated Limousine heifers reared in a farm close to an industrial plant (Grez en Bouère, 53, Mayenne, France). During the first year of their life, the animals were exposed to emissions of organic pollutants, particularly DL-PCBs and NDL-PCBs. In detail, the heifers here studied were reared in an agricultural area near to Aprochim, an industrial plant specialized in treatment and decontamination of equipments, transformers, and oils contaminated by PCBs. The accidentally exposure to pollutants was essentially due to the ingestion of forages contaminated by emissions of the factory Aprochim. This pollution was identified as "PCB contamination" because a control performed by the local prefecture revealed that samples of milk, meat, forages and dairy products, collected in 8 farms located within 3 km from the industrial plant, contained a PCB concentration higher than that legally admitted. The commercialization of meat and dairy products from these farms was then forbidden, and in several farms animals were slaughtered. Indications on the PCB contamination around Aprochim are available online (www.leparisien.fr/.../pcb-trois-elevages-bovins-contamines-abattus-en-m, http://www.vedura.fr/actualite/6945-pollution-pcb-mayenne-troupeaux-abattus-usine-aprochim-mise-demeure; http://www.parismatch.com/Actu/Environnement/Usines-Aprochim-pollueur-vainqueur-595816).

The extent of contamination was evaluated by measuring total toxic equivalent (TEQ) values of DL-PCB + PCDD/F, and NDL-PCB amount in pericaudal fat of each animal. The heifers were removed from the contaminated area (Mayenne, 53) and housed in an experimental facility located far away this area (Meurthe et Moselle, 54, at the "Domaine experimental de la Bouzule", Lorraine University-Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires; Vandoeuvre, France) for six months. The animals were reared under controlled conditions, and fed a mixed ration based on grass silage, hay, straw, soybean and corn, with the objective of a weight gain of 1000 g/d. The animals were weighed, blood sampled and submitted to a pericaudal biopsy to get 2 g fat samples two weeks after their arrival in the experimental facility (day 0, sampling A) and bimonthly during the decontamination period (59, 125 and 188 days after starting the decontamination; samplings B, C, and D respectively). The fat samples were stored at −20 °C before being analysed.

TEQ values of DL-PCBs + PCDD/Fs in pericaudal fat were measured, in order to monitor and evaluate the efficiency of the decontamination procedure. The analyses were carried out on pericaudal fat because pericaudal biopsies are minimally invasive, and the animals rapidly recover. In addition, a correlation between the dioxins level in caudal and internal (perirenal) fat was demonstrated (EU-RL, 2009), and it was proposed that the easily accessible subcutaneous fat represents a reliable estimate of adipose tissue contamination by pollutants (Kim et al., 2011).

Eight one year old Charolaise heifers reared at the "Domaine experimental de la Bouzule (Meurthe et Moselle, 54)", i.e. far away from any known PCBs source, were also included in the study as control group and sampled on December 2011 (sampling K1) and March 2012 (sampling K2). Charolaise heifers can differ from the Limousine heifers for fat body mass, and for other general characteristics, so they were used as "control" only because represent heifers never exposed to PCB pollution and reared in the experimental farm where the decontamination took place.

Blood samples were centrifuged at 500 *g* (20 min; 4 °C), and *sera* were used for measuring the concentration of non-enzymatic antioxidants (Retinol, alpha-Tocopherol, and Ascorbate), and the activities of enzymatic antioxidants, superoxide dismutase (SOD) and glutathione peroxidase (GPx), here used as indices of the antioxidant defence system. The total antioxidant capacity (TAC) was also assessed, as it well reflects the overall antioxidative potential of the whole organism (Kankofer et al., 2010), and effectively describes the equilibrium between pro-oxidants and antioxidants in blood (Ghiselli et al., 2000). Oxidative modifications were monitored by measuring serum concentrations of nitro-tyrosine (N-Tyr) and protein-bound carbonyls (PC), for evaluating the extent of oxidative damage to protein, and level of lipid hydroperoxides (LPOs) for assessing the extent of lipid peroxidation, induced by the interaction of free radicals with polyunsaturated fatty acids. Haptoglobin and TNF-alpha concentrations were measured as inflammation markers.

Protein concentration in each serum sample (previously diluted 1:80 in 130 mM NaCl, 20 mM Tris–HCl, pH 7.4) was measured by Bradford assay (Bradford, 1976).

2.3. DL-PCBs and NDL-PCB analysis

Chemical analyses of PCBs were performed at the French National Reference Laboratory (LABERCA, ONIRIS, Nantes) according to the requirements of the quality assurance parameters of the Commission Directive 2002/69/EC and 2002/70/EC of July 2002 laying down the sampling methods and the methods of analysis for the determination of PCBs in foodstuffs and feeding stuffs, respectively. Moreover, analyses were performed upon an accredited system ISO 17025. All the methods used have been validated and are accredited ISO 17025. Furthermore, this research project was conducted under a certified system ISO 9001 v. 2000 standard. Briefly, fat samples and feed (~2–5 g) were weighed, and 18 ^{13}C-labelled PCBs from Cambridge Isotope Laboratories and Wellington Laboratories were added as internal standards to each

Table 1
PCDD/F + DL-PCB and NDL-PCB levels in pericaudal fat of Limousine heifers under decontamination.

	A	B	C	D
TEQs (PCDD/Fs + DL-PCBs; pg/g of fat)	25.34 ± 1.41***	8.33 ± 0.35.	4.87 ± 0.22	3.13 ± 0.18
NDL-PCBs (ng/g of fat)	165.9 ± 14.42**	56.86 ± 2.88	33.78 ± 1.50	24.16 ± 1.42
Days from starting decontamination	0	59	125	188
Date of sampling (mm, dd, yy)	09/15/11	11/18/11	01/23/12	03/27/12
N	8	8	8	8

Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCBs source. Analysis were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (day 59, 125, 188; B, C, D).

TEQs: toxic equivalents; PCDDs: polychlorinated dibenzo-*para*-dioxins; DL-PCBs: dioxin-like polychlorinated biphenyls; PCDFs: polychlorinated dibenzo-furans; NDL-PCBs: non-dioxin-like polychlorinated biphenyls.

*** $P < 0.001$ vs B, C, or D.

** $P < 0.001$ vs B, C, or D.

Table 2
Profile of fat contamination by PCDDs, PCDFs and DL-PCBs.

	A	B	C	D
Total of PCDDs	0.438 ± 0.033	0.265 ± 0.017	0.210 ± 0.030	0.166 ± 0.021
Total of PCDFs	0.508 ± 0.040	0.353 ± 0.029	0.231 ± 0.017	0.172 ± 0.013
OMS TEQ DL-PCBs	24.39 ± 1.364	7.714 ± 0.323	4.428 ± 0.199	2.763 ± 0.176
Total TEQs (PCDDs/F + PCB DL)	25.34 ± 1.405	8.333 ± 0.353	4.869 ± 0.215	3.102 ± 0.184
Days from starting decontamination	0	59	125	188
N	8	8	8	8

Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCBs source. Analysis were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (day 59, 125, 188; B, C, D).
TEQs: toxic equivalents; PCDDs: polychlorinated dibenzo-*para*-dioxins; DL-PCBs: dioxin-like polychlorinated biphenyls; PCDFs: polychlorinated dibenzo-furans.
Data are expressed as pg per g of pericaudal fat.

sample. The solid samples were extracted by pressurized liquid extraction (ASE 300, Dionex, Sunnyvale, CA, USA), using mixed toluene (Picograde — 1350 Promochem) and acetone (Picograde — 1142 Promochem) at 70/30 (v/v). Final measurement of PCBs was performed by GC–HR–MS (gas chromatograph-HP-5890) from Hewlett Packard (Palo Alto, CA, USA). The mass spectrometer (JMS 700 D, Jeol, Tokyo, Japan) was set at a resolution of 10,000, in electron ionization mode. Single Ion Monitoring (SIM) was used to record the two most abundant signals of the molecular ion (35Cl and 37Cl isotopic contribution). A DB-5MS capillary column (30 m × 0.25 mm i.d.,·0.25 μm film thickness) from J&W was used in splitless mode.

The GC temperature programme was 120 °C (3 min), 20 °C min^{-1} to 170 °C (0 min), 3 °C min^{-1} to 245 °C (0 min) and finally 20 °C min^{-1} to 275 °C (7 min). The GC programme for Signals was integrated by JEOL

Table 3
Congeners analysis.

	A	B	C	D
PCDD				
2,3,7,8-TCDD	**0.091 ± 0.005**	0.057 ± 0.009	0.054 ± 0.006	0.051 ± 0.010
1,2,3,7,8-PeCDD	**0.232 ± 0.030**	0.147 ± 0.012	0.100 ± 0.027	0.070 ± 0.010
1,2,3,4,7,8-HxCDD	0.016 ± 0.001	0.010 ± 0.001	0.013 ± 0.002	0.005 ± 0.0010
1,2,3,6,7,8-HxCDD	**0.087 ± 0.010**	0.036 ± 0.003	0.029 ± 0.002	0.024 ± 0.003
1,2,3,7,8,9-HxCDD	0.014 ± 0.001	0.009 ± 0.001	0.009 ± 0.0005	0.006 ± 0.0005
HpCDD	0.011 ± 0.001	0.007 ± 0.0008	0.005 ± 0.0004	0.012 ± 0.003
OCDD	0.0008 ± 0.0001	0.0007 ± 0.00008	0.0006 ± 0.00004	0.0004 ± 0.00008
Total of PCDDs	0.438 ± 0.033	0.265 ± 0.017	0.210 ± 0.030	0.166 ± 0.021
Days from starting decontamination	0	59	125	188
PCDF				
2,3,7,8-TCDF	0.024 ± 0.006	0.012 ± 0.004	0.005 ± 0.0007	0.022 ± 0.003
1,2,3,7,8-PeCDF	0.003 ± 0.0007	0.007 ± 0.0009	0.003 ± 0.0003	0.002 ± 0.0004
2,3,4,7,8-PeCDF	**0.313 ± 0.019**	0.156 ± 0.017	0.106 ± 0.010	0.085 ± 0.005
1,2,3,4,7,8-HxCDF	**0.080 ± 0.011**	0.092 ± 0.011	0.058 ± 0.004	0.027 ± 0.004
1,2,3,6,7,8-HxCDF	0.039 ± 0.005	0.041 ± 0.004	0.024 ± 0.001	0.017 ± 0.002
1,2,3,7,8,9-HxCDF	0.005 ± 0.0008	0.004 ± 0.0006	0.004 ± 0.0004	0.004 ± 0.0004
2,3,4,6,7,8-HxCDF	0.027 ± 0.002	0.030 ± 0.003	0.019 ± 0.002	0.015 ± 0.001
1,2,3,4,6,7,8-HpCDF	0.015 ± 0.003	0.009 ± 0.001	0.009 ± 0.0006	0.004 ± 0.001
1,2,3,4,7,8,9-HpCDF	0.002 ± 0.0003	0.002 ± 0.0002	0.002 ± 0.0003	0.0008 ± 0.0001
OCDF	0.0005 ± 0.00009	0.0004 ± 0.00006	0.0003 ± 0.00003	0.0002 ± 0.00005
Total of PCDFs	0.508 ± 0.040	0.353 ± 0.029	0.231 ± 0.017	0.172 ± 0.013
OMS TEQ PCDD/F	0.945 ± 0.063	0.619 ± 0.046	0.441 ± 0.040	0.339 ± 0.032
Days from starting decontamination	0	59	125	188
DL-PCB				
PCB77	0.0009 ± 0.0001	0.0007 ± 0.00009	0.0004 ± 0.00004	0.0004 ± 0.00005
PCB81	0.0007 ± 0.00009	0.0002 ± 0.00003	0.0001 ± 0.000009	0.0001 ± 0.00001
PCB126	**22.39 ± 1.236**	7.033 ± 0.296	4.027 ± 0.188	2.468 ± 0.163
PCB169	**1.330 ± 0.129**	0.430 ± 0.026	0.249 ± 0.010	0.186 ± 0.012
Total of coplanares	23.73 ± 1.327	7.465 ± 0.315	4.276 ± 0.196	2.654 ± 0.171
PCB105	0.037 ± 0.003	0.011 ± 0.0009	0.006 ± 0.0005	0.005 ± 0.0004
PCB114	0.006 ± 0.0006	0.003 ± 0.0001	0.002 ± 0.00006	0.001 ± 0.00008
PCB118	**0.398 ± 0.028**	0.160 ± 0.006	0.099 ± 0.004	0.066 ± 0.004
PCB123	0.003 ± 0.0001	0.0012 ± 0.00007	0.0007 ± 0.00004	0.0007 ± 0.00004
PCB156	0.122 ± 0.013	0.040 ± 0.002	0.024 ± 0.001	0.019 ± 0.001
PCB157	0.018 ± 0.002	0.007 ± 0.0004	0.004 ± 0.0002	0.005 ± 0.0004
PCB167	0.062 ± 0.005	0.021 ± 0.001	0.013 ± 0.0004	0.009 ± 0.0006
PCB189	0.0189 ± 0.002	0.006 ± 0.0005	0.003 ± 0.0001	0.002 ± 0.0002
Total of non-coplanares	0.665 ± 0.050	0.249 ± 0.010	0.152 ± 0.006	0.109 ± 0.006
OMS TEQ DL-PCB	24.39 ± 1.364	7.714 ± 0.323	4.428 ± 0.199	2.763 ± 0.176
Total TEQ (PCDD/F + PCB DL)	25.34 ± 1.405	8.333 ± 0.353	4.869 ± 0.215	3.102 ± 0.184
Days from starting decontamination	0	59	125	188

Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCB source. Analysis were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (days 59, 125, 188; B, C, D).
TEQs: toxic equivalents; PCDDs: polychlorinated dibenzo-*para*-dioxins; DL-PCBs: dioxin-like polychlorinated biphenyls; PCDFs: polychlorinated dibenzo-furans.
Data are expressed as pg per g of pericaudal fat.
The more abundant congeners are marked in bold.

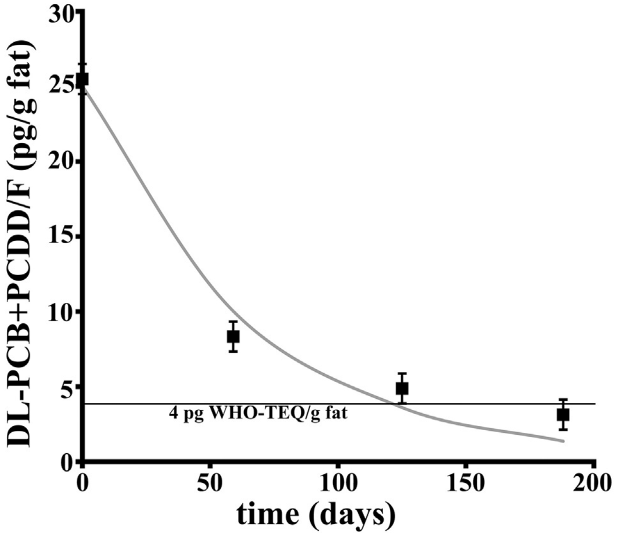


Fig. 1. Decontamination kinetic of DL-PCB and PCDD/F in growing Limousine heifers. Eight one year old Limousine heifers were removed from a contaminated farm, located close to an industrial plant, and housed in an experimental facility located far away this area, for six months, for a decontamination attempt. The extent of individual contamination was evaluated by measuring total toxic equivalent (TEQ) values of DL-PCBs + PCDD/Fs in pericaudal fat of each animal at the start of the decontamination process (day 0), and bimonthly during the decontamination period (days 59, 125 and 188). Data are reported as mean ± standard deviation. The threshold limit for DL-PCB + PCDD/F (4 pg WHO-TEQ/g fat; European Union Regulation No 1259/2011) is indicated by a continuous line.

Diok V2 software. All these values were automatically corrected by taking into account the recovery rate of the ^{13}C labelled molecules. The PCBs detection limits in the different tissues and serum analysed including all congeners were better than 30 fg g^{-1} of the matrix.

2.4. Determination of antioxidants and lipid hydroperoxides (LPOs)

Ascorbate (Asc) concentration in serum samples was measured by HPLC, using the anion exchange column Nucleosil 100-NH$_2$, as previously described (Spagnuolo et al., 2011). Retinol (Ret) and alpha-Tocopherol (Toc) levels were titrated by HPLC, using the reverse phase C18 column Nova-PAK C18, according to a published procedure (Spagnuolo et al., 2011). The total antioxidant capacity (TAC) was measured by the Trolox Equivalent Antioxidant Capacity Assay, according to Miller et al. (1993). Samples were reacted with the radical 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) [ABTS], and the antioxidant capacity was measured as change of the absorbance at 734 nm, and expressed as μM concentration of Trolox equivalents (Spagnuolo et al., 2001).

LPO concentration was measured by a colorimetric quantitative assay, using the Lipid Hydroperoxide Assay Kit of Cayman Chemical, according to the manufacturer's instructions.

2.5. Determination of nitro-tyrosine (N-Tyr)

Nitrated protein levels in serum samples were measured by ELISA, essentially according to a published procedure (Cigliano et al., 2014). Briefly, samples were diluted (1:500, 1:1500, 1:3000, and 1:9000) with coating buffer (7 mM Na$_2$CO$_3$, 17 mM NaHCO$_3$, 1.5 mM NaN$_3$, pH 9.6), and incubated in the wells of a microtitre plate overnight at 4 °C. Standard curves were obtained with serial dilutions of nitrated BSA. N-Tyr was detected by incubation with Rabbit anti-N-Tyr antibody (1:1500 dilution in 130 mM NaCl, 20 mM Tris–HCl, 0.05% Tween 20, pH 7.3, supplemented with 0.25% BSA; 1 h, 37 °C), followed by GAR-HRP (1:3000 dilution). Colour development was monitored at 492 nm. Data were reported as nmol of N-Tyr per mg of proteins.

2.6. Determination of protein-bound carbonyls

PC concentration in serum was titrated by ELISA. Protein derivatization, sample dilution, and immunodetection with Rabbit anti-DNP antibody were carried out according to Cigliano et al. (2014). Data were reported as nmol of carbonyls per mg of proteins.

2.7. Evaluation of glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities

GPx activity was measured indirectly by a coupled reaction with glutathione reductase (GR), using the glutathione peroxidase assay kit of Cayman Chemical, according to the manufacturer's instructions. GPx activity was expressed as nmol of NADPH oxidized per minute per ml of sample. SOD activity was measured with the superoxide dismutase assay kit of Cayman Chemical, according to the manufacturer's instructions. SOD activity was expressed Unit/ml. One unit of SOD is defined as the amount of the enzyme needed to exhibit 50% dismutation of the superoxide radical.

2.8. Haptoglobin (Hpt) and TNF-alpha titration

Hpt concentration in individual serum samples was measured by ELISA (Spagnuolo et al., 2014). Samples were diluted (1:4000, 1:8000; 1:16,000) with coating buffer, and incubated in the wells of a microtitre plate (overnight, 4 °C). Hpt was detected by Rabbit anti-Hpt IgG (1:1500 dilution; 1 h, 37 °C), followed by GAR-HRP (1:4500 dilution, 1 h, 37 °C). The calibration curve was obtained by assaying the immunoreactivity of 6, 3, 1.5, 1, 0.75, 0.5, 0.25 ng of commercial Hpt standard.

TNF-alpha concentration was titrated by sandwich ELISA. Aliquots (50 μl; 12 μg/ml) of mouse anti-bovine TNF-alpha IgG were coated into the wells of a microtitre plate (2 h, room temperature). The plate was extensively washed and blocked (1 h, 37 °C) by incubation with 1% BSA in PBS (140 mM NaCl, 10 mM Na$_2$HPO$_4$, 2 mM KH$_2$PO$_4$, 2.7 mM KCl). Then aliquots (50 μl) of serum samples (dilution 1:10, 1:30 in PBS) were incubated into the wells (overnight, 4 °C). TNF-alpha was detected by incubation with biotinylated mouse anti-

Table 4
NDL-PCB congener analysis.

	A	B	C	D
PCB28	0.276 ± 0.030	0.206 ± 0.035	0.104 ± 0.010	0.061 ± 0.010
PCB52	0.231 ± 0.039	0.217 ± 0.039	0.088 ± 0.011	0.118 ± 0.019
PCB101	0.388 ± 0.044	0.318 ± 0.057	0.143 ± 0.020	0.203 ± 0.032
PCB138	**48.57 ± 4.136**	16.80 ± 1.012	10.12 ± 0.451	6.738 ± 0.446
PCB153	**70.26 ± 5.742**	23.92 ± 1.176	14.59 ± 0.668	10.43 ± 0.567
PCB180	**46.29 ± 5.167**	15.43 ± 0.818	8.733 ± 0.441	6.614 ± 0.456
Total of NDL-PCBs	165.9 ± 14.42	56.86 ± 2.884	33.78 ± 1.502	24.16 ± 1.422
Days from starting decontamination	0	59	125	188
N	8	8	8	8

Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCBs source. Analysis were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (days 59, 125, 188; B, C, D).
The more abundant congeners are marked in bold.

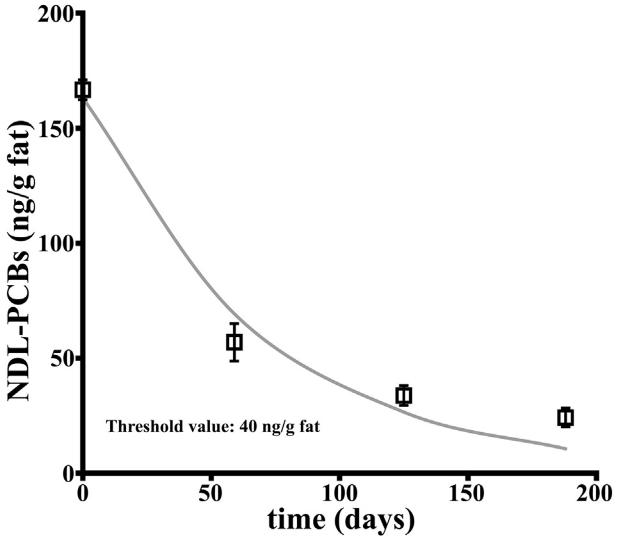


Fig. 2. Decontamination kinetic of NDL-PCBs in growing Limousine heifers. Eight one year old Limousine heifers were removed from a contaminated farm, located close to an industrial plant, and housed in an experimental facility far away this area, for six months, for a decontamination attempt. The extent of individual contamination was evaluated by measuring NDL-PCBs amount in pericaudal fat of each animal at the start of the decontamination process (day 0), and bimonthly during the decontamination period (days 59, 125, and 188). Data are reported as mean ± standard deviation. The threshold limit for NDL-PCBs (40 ng WHO-TEQ/g fat; European Union Regulation No 1259/2011) is indicated.

TNF-alpha IgG (1:100 dilution; 2 h, 37 °C) followed by treatment with Streptavidin–HRP (1:1500 dilution; 1 h, 37 °C). The calibration curve was obtained by assaying the immunoreactivity of 20, 10, 5, 2.5, 1, 0.5 pg of recombinant bovine TNF-alpha.

2.9. Statistical analysis

The time effect linked to the PCB levels in fat was analysed using the MIXED procedure of SAS, the individual animal being the experimental unit. The student t test was used to compare the mean values between time points.

The samples for measurement of SOD and GPx activities, PC, N-Tyr, Hpt, TNF-alpha, or LPO concentration were processed in triplicate. The titration of Toc, Ret, and Asc was carried out on duplicates. Values were expressed as mean ± SEM. Significance of statistical differences was evaluated by one-way ANOVA, followed by Dunnett's test, for comparing data from samplings performed at days 59, 125 and 188 to data from sampling performed at day 0, or by Tukey's test for multiple comparisons, using the GraphPad Prism 5.01 programme (Graph Pad Software, San Diego, CA, USA). Differences were considered statistically significant when the two-sided P value was less than 0.05. The GraphPad Prism 5.01 programme was also used to calculate correlation coefficient (Pearson's r).

3. Results

3.1. Animal growing performances and contamination profile in pericaudal fat

During the 6-month experimental period, the average live weight of the animals increased from 337 to 524 kg, i.e. a mean daily weight of about 1000 g/d. This weight gain was in line with expectations for one year old Limousine heifers.

The analysis of PCDD, PCDF, DL-PCBs, and NDL-PCBs was carried out on pericaudal fat samples essentially because the European Union Regulation indicated Maximum Residue Levels of PCB legally admitted in bovine fat. In addition, the amount of PCBs stored in adipose tissue occurs as a result of bioaccumulation of these toxins over a lifetime of exposure, and it was suggested that the measurement of fat-adjusted values accurately describes the total burden of PCBs residing in adipose tissue through- out the body (Patterson et al., 1988).

As shown in Table 1, both DL-PCBs and NDL-PCBs significantly contributed to bovine contamination. In particular, at the start of the experimental period (time 0, sampling A; two weeks after the removal of the animals from the contaminated area and their arrival to the uncontaminated experimental farm) TEQ value of DL-PCBs and PCDD/Fs was 25.34 ± 1.405 pg/g fat (Table 1). This value was about 6 fold higher than that legally permitted, fixed at 4 pg/g fat (European Union Regulations No 1881/2006, 1259/2011). DL-PCBs provided by far the highest contribution to the TEQ value (about 96%; Table 2), while PCDD/Fs represented less than 4% of the total TEQ (0.945 ± 0.063 pg/g of fat). The high proportion of DL-PCBs was probably due to the origin of the contamination (ingestion of forages contaminated by the emissions of an industrial plant specialized in treatment and decontamination of PCB).

The analysis of contamination profile (Table 3) demonstrated that the more abundant congener among DL-PCBs was PCB 126 (92% of the DL-PCBs), followed by PCB 169 (5.5%) and PCB 118 (1.6%). In addition, the more represented congeners among PCDD/Fs were 2,3,4,7,8-PeCDF (33%), and 1,2,3,7,8-PeCDD (25%), followed by 2,3,7,8-TCDD (9.6%), 1,2,3,6,7,8-HxCDD (9.2%) and 1,2,3,4,7,8-HxCDF (8.5%).

Depletion curve of DL-PCB + PCDD/Fs (Fig. 1) is described by the equation $y = 20.522e^{-0.011x}$, and demonstrates that half life of these compounds is 44 days. Further, 148 days were necessary to reach the threshold value for DL-PCB + PCDD/Fs (4 pg WHO-TEQ/g fat), and at the end of the experimental period, all animals were considered decontaminated, as TEQ value in pericaudal fat was lower than that legally admitted (European Union Regulation No 1259/2011). Depletion curves of more abundant congeners among PCDD/Fs are shown in

Table 5
Markers of the antioxidant defence system in serum of heifers.

	A	B	C	D	K1	K2
Ret (μg/ml)	0.48 ± 0.02**	0.72 ± 0.02	0.73 ± 0.01	0.68 ± 0.01	0.71 ± 0.01***	0.71 ± 0.08***
Toc (μg/ml)	1.48 ± 0.04**	1.73 ± 0.02	1.76 ± 0.03	1.77 ± 0.03	1.85 ± 0.03***	1.87 ± 0.03***
Asc (μM)	6.06 ± 0.11**	7.76 ± 0.20	7.95 ± 0.16	8.01 ± 0.12	8.00 ± 0.11***	8.42 ± 0.27***
GPx (nmol/min/ml)	95.73 ± 4.08**	164.1 ± 6.7	169.2 ± 8.5	167.0 ± 8.3	170.2 ± 15***	181.1 ± 14***
SOD (U/ml)	1.10 ± 0.04**	1.55 ± 0.06	1.57 ± 0.04	1.62 ± 0.04	1.67 ± 0.06***	1.69 ± 0.09***
TAC (μM)	92.24 ± 2.76**	133.0 ± 3.5	139.4 ± 4.2	141.0 ± 3.8	139.9 ± 3.2***	146.7 ± 2.6***
Days from starting decontamination	0	59	125	188		
Date of sampling (mm, dd, yy)	09/15/11	11/18/11	01/23/12	03/27/12	12/15/11	03/29/12
N	8	8	8	7	8	6

Ret, Retinol; Toc, α-Tocopherol; Asc, Ascorbate; TAC, total antioxidant capacity; GPx, glutathione peroxidase activity; SOD, superoxide dismutase.
Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCBs source. Samplings were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (days 59, 125, 188; B, C, D).
K1 and K2 are samplings, taken at different times, from Charolaise heifers reared in the same experimental farm far away from any PCBs source, and regarded as controls.
*** A vs K1 or K2, P < 0.001.
** A vs B, C, or D, P < 0.001.

Supplementary Fig. S1. Depletion curves of more abundant congeners among DL-PCBs are shown in Supplementary Fig. S2. Half lives of PCB 126, PCB 169 and PCB 118 were calculated and they were found to be 43, 40, and 59 days respectively.

NDL-PCB concentration at the beginning of the decontamination period (165.9 ± 14.42 ng/g of fat) was about 4 fold higher than that legally admitted (Table 1), thus strongly contributing to bovine contamination. As shown in Table 4, the highest contribution to NDL-PCB contamination was from PCB 153 (42%), PCB 138 (29%), and PCB 180 (28%). Depletion curve of NDL-PCB (Fig. 2), described by the equation $y = 132.87e^{-0.01x}$, demonstrates that half life of these compounds is 47 days, and the threshold value of NDL-PCBs (40 ng/g of fat) was reached within 120 days. The specific contamination profile (Table 4) demonstrated that the more abundant congeners were PCB 153 (42.4% of total; calculated half life 47 days), followed by PCB 138 (29.3%; calculated half life 49 days) and PCB 180 (27.9%; calculated half life 46 days). Depletion curves of each congener are shown in Supplementary Fig. S3.

3.2. Analysis of serum antioxidants and total antioxidant capacity

Serum concentrations of Ret, Toc, and Asc, the total antioxidant capacity (TAC), as well as GPx and SOD activities were measured as markers of the antioxidant defence system (Table 5).

Ret concentration was positively correlated with Toc concentration only in control animals ($r = 0.805$, $P = 0.016$, sampling K1; $r = 0.930$, $P = 0.007$, sampling K2), and in the samplings performed 125 (sampling C) and 188 (sampling D) days after starting the decontamination process (C, $r = 0.752$, $P = 0.027$; D, $r = 0.769$, $P = 0.035$). As depicted in Table 5, the concentrations of Toc, Ret, and Asc were significantly lower in samples collected at the start of experimental period (day 0; sampling A) with respect to either the control (Toc, 25.88 ± 0.47%; Ret, 47.40 ± 0.21%; Asc, 35.50 ± 3.51%; $P = 0.001$) or to animals sampled during the decontamination period (Toc, 18.54 ± 0.78%; Ret, 47.82 ± 3.07%; Asc, 30.51 ± 1.27%; $P = 0.001$). No statistically significant differences between values measured in samples from control cows and those collected from animals undergoing decontamination (sampling B, C, or D) were recorded.

Similarly the TAC, and the activities of SOD and GPx were significantly more elevated (55.36 ± 3.69%, 53.05 ± 0.87%, and 83.48 ± 5.69% respectively; $P = 0.001$) in serum from control cows than in samples taken at the beginning of decontamination (A), and no differences between control samplings and samplings taken during decontamination (B, C and D) were revealed.

A positive correlation between TAC and Asc concentration was found in control cows and in animals undergoing decontamination (K1, $r = 0.853$, $P = 0.007$; K2, $r = 0.868$, $P = 0.025$; B sampling, $r = 0.793$, $P = 0.019$; C sampling, $r = 0.787$, $P = 021$; D, $r = 0.912$, $P = 0.004$; data not shown), but not in cows sampled at time 0 (sampling A). Likewise, Asc concentration was positively correlated with SOD activity in unexposed cows and in cows fed the decontamination diet (K1, $r = 0.818$, $P = 0.013$; K2, $r = 0.823$, $P = 0.040$; B, $r = 0.732$, $P = 0.039$; C, $r = 0.876$, $P = 004$; D, $r = 0.852$, $P = 0.015$; data not shown). Finally TAC was negatively correlated with total TEQ of DL-PCBs + PCDD/Fs ($r = -0.8523$, $P = 0.007$; Fig. 3, panel A), and with NDL-PCBs ($r = -0.789$, $P = 0.035$; Supplementary Fig. S4, panel A) in samples collected at the beginning of experimental period.

3.3. Analysis of serum PC, N-Tyr and LPO

As reported in Table 6, serum levels of N-Tyr and PC were found significantly higher in sampling performed at time 0 (A) than in those from control animals (40.75 ± 0.38% and 44.78 ± 1.38% respectively; $P = 0.001$), or in those taken during the decontamination period (33.35 ± 2.73% and 43.80 ± 1.98% respectively; $P = 0.001$). In addition, the level of N-Tyr was higher (17.43 ± 0.53%; $P < 0.05$) in samples collected 59 days after the start of decontamination process (sampling B) than in samplings from unexposed animals, and it was also higher (11.66 ± 1.36%; $P < 0.05$) with respect to the samplings performed after 125 (sampling C) and 188 days (sampling D) of decontamination. Conversely, the level of PC did not differ among these groups. When compared

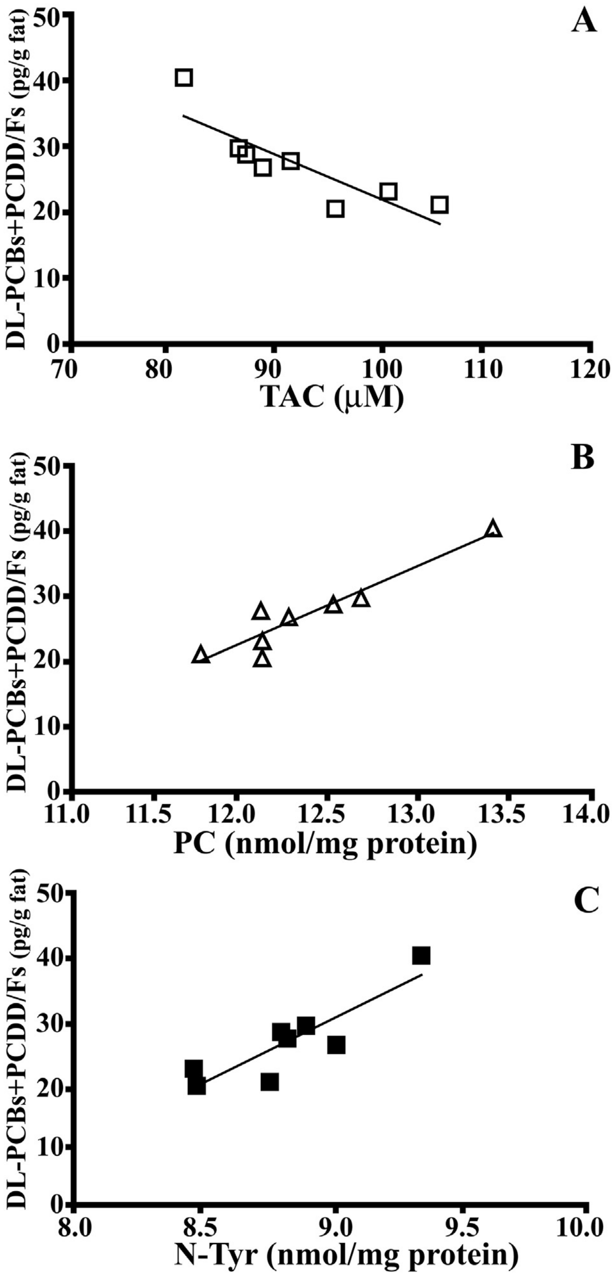


Fig. 3. Correlation between total TEQ in pericaudal fat and specific serum markers of redox homeostasis. Panel A. The total antioxidant capacity (TAC) was measured in serum samples from Limousine heifers at the starting point of decontamination (day 0), and expressed as μM concentration of Trolox equivalents. Each sample was analysed in triplicate, and the average value was calculated. Deviations over 5% from the mean were not found. Total toxic equivalent (TEQ) values of DL-PCBs + PCDD/F in pericaudal fat of each animal was measured and reported as pg/g fat. The statistical programme Graph Pad Prism 5.01 performed Pearson correlation analysis and the calculation of P ($r = -0.8523$, $P = 0.007$). Panel B. The level of PC was measured, by ELISA, in serum samples collected at the starting point of decontamination (day 0), and expressed as nmol/mg of protein. The statistical programme Graph Pad Prism 5.01 performed Pearson correlation analysis and the calculation of P ($r = 0.942$, $P = 0.0005$). Panel C. The concentration of N-Tyr was measured, by ELISA, in serum samples collected at the starting point of decontamination (day 0), and expressed as nmol/mg of protein. The statistical programme Graph Pad Prism 5.01 performed Pearson correlation analysis and the calculation of P ($r = 0.880$, $P = 0.004$).

with serum samples collected at time 0 (sampling A), statistically significant lower LPO concentrations were detected in the control cows (51.11 ± 0.35%; P = 0.001), as well as in cows under decontamination (44.09 ± 1.25%; P = 0.001). No differences between control samplings and samplings taken during decontamination (B, C and D) were observed.

Serum titre of N-Tyr was negatively correlated with SOD activity, Asc concentration, and TAC in both samplings from control cows, namely K1 (SOD, r = −0.790, P = 0.020; Asc, r = −0.866, P = 0.006; TAC, r = −0.718, P = 0.045), and K2 (SOD, r = −0.885, P = 0.019; Asc, r = −0.906, P = 0.013; TAC, r = −0.898, P = 0.015). Further, the concentration of N-Tyr was negatively correlated with TAC and SOD activity both in contaminated heifers (sampling A, r = −0.736, P = 0.037; r = −0.765, P = 0.027, respectively) and in sera collected during the decontamination i.e. B (r = −0.0718, P = 0.041; r = −0.853, P = 0.007, respectively), C (r = −0.899, P = 0.002; r = −0.784, P = 0.042, respectively), and D (r = −0.950 P = 0.001; r = 0.756, P = 0.043).

Finally, both PC and N-Tyr concentrations were correlated with total TEQ of DL-PCBs + PCDD/Fs (sampling A, r = 0.942, P = 0.0005; r = 0.880, P = 0.004 respectively; Fig. 3, panels B and C, respectively) and with NDL-PCBs (r = 0.806, P = 0.029; r = 0.842, P = 0.018 respectively; Supplementary Fig. S4, panels B and C, respectively) in samples obtained at the start of decontamination.

3.4. Analysis of Hpt and TNF-alpha

As shown in Fig. 4, serum Hpt concentration was found lower in samples collected at the start of decontamination (A) compared with samplings from control cows (111.9 ± 1.66%; P < 0.001), and with samplings taken during the decontamination phase (80.88 ± 7.45%; P < 0.01). No statistically significant differences among control animals and animals undergoing decontamination were found.

TNF-alpha concentration was significantly higher in samples collected at time 0 (A) and after 59 days of decontamination (B) than in those from control animals (A, 46.06 ± 3.44%, P < 0.001; B, 35.30 ± 4.13%, P < 0.05), or from samplings taken after 125 (C) and 188 (D) days of decontamination (A, 41.46 ± 3.08%, P < 0.001; B, 29.77 ± 3.69%, P < 0.05) (Fig. 4). Values from control cows and from samplings C and D did not significantly differ. Further, Hpt concentration was correlated with total TEQ of DL-PCBs + PCDD/Fs (Fig. 5) and with NDL-PCBs (Supplementary Fig. S5) in both sampling A (DL-PCBs + PCDD/Fs, r = −0.735, P = 0.038, Fig. 5, panel A; NDL-PCBs, r = −0.913, P = 0.004, Supplementary Fig. S5, panel A), and in sampling B (DL-PCBs + PCDD/Fs, r = −0.946, P = 0.0004, Fig. 5, panel B; NDL-PCBs, r = −0.763, P = 0.028, Supplementary Fig. S5, panel B). Similarly, TNF-alpha level correlated with total TEQ of DL-PCBs + PCDD/Fs (Fig. 5) and with NDL-PCBs (Supplementary Fig. S6) in both sampling A (DL-PCBs + PCDD/Fs, r = 0.881, P = 0.004, Fig. 5, panel A; NDL-PCBs, r = 0.895, P = 0.007, Supplementary Fig. S6, panel A) and in sampling B (DL-PCBs + PCDD/Fs, r = 0.791, P = 0.019, Fig. 5, panel B; NDL-PCBs, r = 0.756, P = 0.03, Supplementary Fig. S6, panel B).

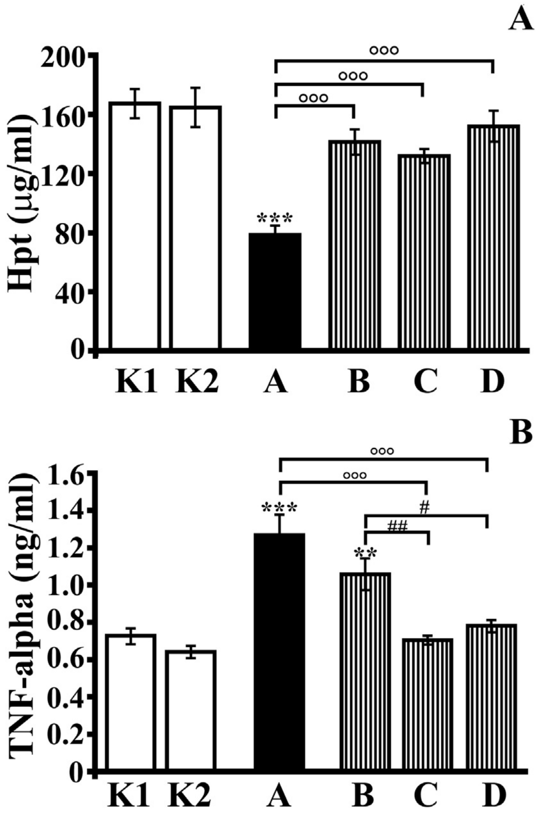


Fig. 4. Serum concentration of Hpt and TNF-alpha. Hpt (panel A) and TNF-alpha (panel B) concentration in serum was measured by ELISA. K1 and K2 (open bar), samplings from Charolaise heifers reared in the experimental farm far away from any PCBs source, here regarded as controls. A (full bar), sampling from Limousine heifers exposed to organic pollutants collected at the start of the experimental period (day 0). B, C and D (bar with vertical lines), samplings performed after 59 (B), 125 (C), and 188 (D) days of decontamination. °°°P < 0.001 vs A (from Dunnett's test). #P < 0.05 vs B; ##P < 0.01 vs B (from Tukey's test). **P < 0.01 vs K1 and K2; ***P < 0.001 vs K1 and K2 (from Tukey's test).

4. Discussion

This study was carried out on a group of Limousine heifers that were accidentally exposed to environmental pollutants by the ingestion of forages contaminated by the emissions of an industrial plant, specialized in treatment and decontamination of equipments, transformers, and oils contaminated by PCBs. The pollution was identified as "PCBs

Table 6
Markers of oxidative stress in serum of heifers.

	A	B	C	D	K1	K2
PC (nmol/mgP)	12.39 ± 0.17**	7.29 ± 0.32	7.11 ± 0.15	6.48 ± 0.44	6.67 ± 0.22***	7.01 ± 0.31***
N-Tyr (nmol/mgP)	8.82 ± 0.09**	6.33 ± 0.19	5.50 ± 0.21	5.80 ± 0.36	5.26 ± 0.18***	5.19 ± 0.19***
LPO (μM)	5.83 ± 0.16**	3.16 ± 0.21	3.21 ± 0.24	3.40 ± 0.29	2.87 ± 0.18***	2.83 ± 0.24***
Days from starting decontamination	0	59	125	188		
Date of sampling (mm, dd, yy)	09/15/11	11/18/11	01/23/12	03/27/12	12/15/11	03/29/12
N	8	8	8	7	8	6

Eight one year old Limousine heifers exposed to organic pollutants were removed from the contaminated area and housed in an experimental farm far away from any PCBs source. Samplings were carried out at the start of the experimental period (day 0; A), and then bimonthly through a six months period (days 59, 125, 188; B, C, D).

K1 and K2 are samplings, taken at different times, from Charolaise heifers reared in the same experimental farm far away from any PCB source, and regarded as controls.

PC, protein-bound carbonyls (nmol per mg of protein); N-Tyr, nitro-tyrosine (nmol per mg of protein); LPO, lipid hydroperoxides (μM).

*** A vs K1 or K2, P < 0.001.

** A vs B, C, or D, P < 0.001.

contamination” by a control performed by the local prefecture. The heifers were removed from the contaminated area and housed in an experimental facility located far away this area for a decontamination attempt.

In this context it is worth mentioning that DL-PCBs and NDL-PCBs exert numerous and different toxic effects. DL-PCBs act essentially via binding to AhR (Alsharif et al., 1994; Mandal, 2005), that leads to activation of CYP1A and CYP1B genes (Safe, 1986; Whitlock, 1990; Machala et al., 1998; Matsumura, 2003; Guruge et al., 2009). NDL-PCBs have little or no affinity to the AhR, bind to or activate the constitutive androstane receptor and/or the pregnane X receptor, leading to induction of other CYP enzymes (Waxman, 1999; Masahiko and Honkakoski, 2000; Kliewer, 2003), and display a different toxicological profile (Westerink, 2014). Despite the differences in toxicological profile and mechanisms of action, both DL-PCBs and NDL-PCBs are known to induce oxidative stress and inflammatory response (Dutta et al., 2012; Wens et al., 2011; Ferrante et al., 2015; Westerink, 2014; Abella et al., 2015).

Our goal was to evaluate the changes in specific markers of blood redox homeostasis and inflammation during the decontamination process. To this aim, we carried out a longitudinal investigation by analysing serum and fat samples collected during a six month period (days 0, 59, 125 and 188). Serum samples were used for assessing oxidative and inflammatory status, fat samples for defining the extent of contamination at each time point. We also characterized redox and inflammatory status of eight Charolaise heifers reared in the experimental farm where the decontamination took place. Although Charolaise differ from Limousine heifers for general characteristics, we regarded them as “control” group only because they never ingested contaminated forages.

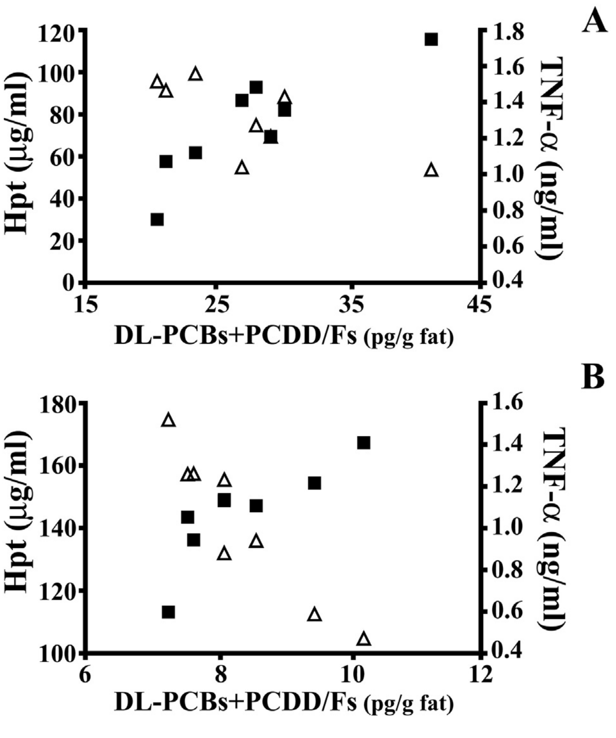


Fig. 5. Correlation between total TEQ in pericaudal fat and serum concentration of Hpt or TNF-alpha. Panel A. Hpt (open triangles) and TNF-alpha (full squares) concentrations were measured, by ELISA, in serum samples from heifers at the start of decontamination (day 0). Each sample was analysed in triplicate, and the average value was calculated. Deviations over 5% from the mean were not found. Total toxic equivalent (TEQ) values of DL-PCBs + PCDD/F in pericaudal fat of each animal are reported as pg/g fat. The statistical programme Graph Pad Prism 5.01 performed Pearson correlation analysis and the calculation of P (Hpt, $r = -0.735$, $P = 0.038$; TNF-alpha, $r = 0.881$, $P = 0.004$ respectively). Panel B. Hpt (open triangles) and TNF-alpha (full squares) concentrations were measured, by ELISA, in serum samples collected 59 days after starting decontamination. The statistical programme Graph Pad Prism 5.01 performed Pearson correlation analysis and the calculation of P (Hpt, $r = -0.946$, $P = 0.0004$; TNF-alpha, $r = 0.791$, $P = 0.019$).

At the start of experimental period (day 0), TEQ values of DL-PCBs + PCDD/PCDF and NDL-PCB amount in pericaudal fat were 6 and 4 fold higher, respectively, than those legally admitted, so bovine were classified as “exposed” to PCBs. DL-PCBs represented about 96% of the total TEQ, and PCB 126 was the more abundant congener detected, with an estimated half life of 43 days. The highest contribution to NDL-PCB contamination was found to be from PCB 153, PCB 138, and PCB 180, whose half lives were 47, 49 and 56 days respectively. The initial value of both DL- and NDL-PCB progressively decreased during the examined period (3 fold at day 59, 5 fold at day 125, 8 fold at day 188), thus indicating that the procedure of decontamination was effective. Threshold values of DL-PCB + PCDD/Fs and NDL-PCBs were reached within 148 days and 120 days respectively, therefore at the end of the experimental period (188 days) bovine were considered decontaminated. In the experimental period a significant weight gain occurred (184.0 ± 9.82 kg, $P < 0.0001$), thus suggesting that decontamination depended on both a dilution process and the excretion of the pollutants from the animal organism.

The blood redox status of the cows was characterized by evaluating specific indices of the antioxidant defence system and oxidative damage to lipids and proteins. In line with previously published data (Hassoun et al., 2002; Slezak et al., 2000; Lai et al., 2010; Spagnuolo et al., 2012), serum concentrations of Ret, Toc and Asc, as well as the TAC and the activities of both SOD and GPx were found significantly lower (about 47%, 26%, 36%, 55%, 53% and 83%) in samples collected at day 0 from PCB contaminated heifers than in control heifers. The levels of antioxidants significantly increased (about 30%) as decontamination goes on, and were found similar to those of unexposed bovine just after 59 days.

Ret and Toc are dietary liposoluble antioxidants, and their serum concentrations were expected to be correlated (Olmedilla et al., 1997; Cigliano et al., 2014). Actually, a correlation was found only in serum samples collected in the last phase of the experimental period (days 125 and 188), when both DL- and NDL-PCB concentrations in fat were within the legal limits, and namely 5–8 fold lower than those measured at time 0. Interestingly, Asc concentration was positively correlated with both TAC and SOD activity in samples collected during decontamination, irrespective of the sampling time (day 59, 125, or 188), but not in the samples obtained at time 0. Taken together, these findings suggest that a severe perturbation of the blood redox homeostasis occurred before starting the decontamination, and that the restoration of physiological concentrations of liposoluble antioxidants might occur more slowly than the other antioxidants here analysed. As expected, a greater extent of oxidative modifications to protein and lipid fractions was detected at the beginning of the experimental period than during decontamination. In particular, PC and LPO concentrations were remarkably higher at day 0 with respect to any other sampling of the decontamination period and of the control cows. As LPO are produced by the oxidative attack of free radicals on polyunsaturated fatty acids, and PC may be generated by reactions of proteins with aldehydes originated during lipid peroxidation processes (Uchida and Stadtman, 1993), our results suggest that lipid peroxidation and its intermediates might play a crucial role in determining oxidative modifications during acute exposure environmental pollutants, here assessed by the concentrations of DL- and NDL-PCBs. Interestingly, the levels of N-Tyr, which is considered the footprint of protein oxidative damage induced by peroxynitrite correlated well with the concentrations of DL- and NDL-PCBs. Indeed serum N-Tyr concentration was just reduced of about 28% after 59 days of decontamination procedure. Further, N-Tyr concentration in samples obtained at day 125 and 188, was significantly lower not only than that at day 0, but also than that at day 59, when TEQ and NDL-PCB concentrations were still over the legal limit (Table 1). Therefore, N-Tyr could represent a useful index for monitoring the decontamination procedure. Finally, TAC, PC and N-Tyr levels correlated with fat content of both DL-and NDL-PCBs only at the start of decontamination.

So we suggested that such parameters could contribute to identify animal exposed to these pollutants, to evaluate the extent of animal exposure, and to monitoring the decontamination process.

TNF-alpha is a proinflammatory multifunctional cytokine released in response to several stress signals (Chen and Goeddel, 2002; Wajant et al., 2003). TNF-alpha plays an important role in acute dioxin-induced toxicity, and, although the cross-talk between TNF-alpha and dioxin signalling cascades is a complex, cell-type specific regulated process (Haarmann-Stemmann et al., 2009), studies in mice, sheep, and guinea pigs demonstrated that the exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin was associated with the increase of serum TNF-alpha levels (Gasiewicz and Neal, 1979; Clark et al., 1991; Moos et al., 1997). In addition, it was recently reported that NDL-PCBS induce TNF-alpha production (Ferrante et al., 2015). Accordingly, and similar to the trend observed for N-Tyr, higher levels of serum TNF-alpha were detected in samplings performed at days 0 (about 44%) and 59 (about 33%) as compared to those obtained at days 125 and 188, suggesting that the observed variations of this cytokine might be associated with changes of DL- and NDL-PCBs. In line with this hypothesis, TNF-alpha level correlated with DL- and NDL-PCB amounts measured at time 0 and after 59 days of decontamination.

Hpt is an acute phase protein with innate antioxidant and immunomodulatory activity, and also acts as molecular chaperone, inhibiting the inappropriate self-association of proteins induced by oxidation or heat (Saeed et al., 2007; Quaye, 2008). Hpt concentration in plasma was reported to be down-regulated in humans exposed to polycyclic aromatic hydrocarbons or to 2,3,7,8,-tetrachlorodibenzo-*p*-dioxins (Kim et al., 2004). Our investigation revealed that Hpt concentration was significantly lower in serum samples collected at time 0 than in those collected during the decontamination. More to the point, serum Hpt was negatively correlated with fat levels of DL- and NDL-PCBs measured at day 0 and 59, thus supporting the hypothesis that the extent of down-regulation of this protein depends on the degree of exposure to pollutants, as assessed by PCBs concentration in pericaudal fat. As Hpt stably binds free Haemoglobin, thus preventing Haemoglobin-related oxidative damage, and also limits the release of haem, which exacerbates oxidative stress (Quaye, 2008), the lower Hpt concentration measured at time 0, when bovine are considered "exposed" to pollutants, might further contribute to the reduction of effectiveness of the antioxidant body defences.

Although the group of Charolaise represents a control only because never exposed to pollution as the Limousine heifers here examined, the finding of significant differences between the two groups, at time 0, led us to suppose that exposure to PCBs, confirmed by the measurements in fat samples, might induce remarkable physiological changes. As oxidative stress contributes to health disorders, and affects zootechnical and/or reproductive performance of dairy cows (Lomba, 1996; Bernabucci et al., 2002, 2005; Castillo et al., 2005; Wilde, 2006; Wathes et al., 2012), the perturbation of oxidative status might have negative implications for animal health and reproduction, and might compromise animal welfare. Interestingly our results demonstrate that a decontamination procedure, based on the feeding a controlled diet, is effective within 148 days, and is able to restore the blood homeostasis.

It is worth to underline that the blood parameters analysed in our study may be affected by oxidative stress induced by different kinds of pollutants, such as sulphur oxides, nitrogen oxides, hydrocarbons, carbon monoxide, and transition metals. However, we propose that, in the limits of a contamination characterized by measuring DL- and NDL-PCBs, serum TAC, N-Tyr, PC, Hpt and TNF-alpha levels could represent useful tools for identifying exposed animals, because their levels correlate with PCB concentration at the starting point of investigation. We report here, for the first time, that the serum concentrations of two inflammatory markers, Hpt and TNF-alpha, correlate with TEQ value and NDL-PCB amount not only at time 0, but also during decontamination, until PCB concentrations are higher than those legally permitted (day 59). So we propose that Hpt and TNF-alpha, in our experimental conditions, could be considered reliable markers of animal exposure. Finally, we propose that N-Tyr and TNF-alpha, whose serum concentrations still differ from those of control animals until fat TEQ and NDL-PCBs are over the legal limit, could be used as biological monitoring markers of the decontamination procedure.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.10.104.

Acknowledgments

The study was supported by CISIA-VARIGEAV project (Caratterizzazione e valorizzazione delle risorse genetiche animali e vegetali della Campania e della Sardegna finalizzate allo sviluppo della filiera bufalina mediante approcci multidisciplinari ed innovativi; grant number 191/2009), and by POR FESR 2007/2013 project (DEDICA: Sviluppo di tecniche biomolecolari di screening per la messa in evidenza dell'esposizione a diossine e sostanze diossino-simili in vacche da latte).

References

Abella, V., Santoro, A., Scotece, M., Conde, J., López-López, V., Lazzaro, V., et al., 2015. Non-dioxin-like polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) induce chondrocyte cell death through multiple pathways. Toxicol. Lett. 234, 13–19.

Alsharif, N.Z., Lawson, T., Stohs, S.J., 1994. Oxidative stress induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin is mediated by the aryl hydrocarbon (Ah) receptor complex. Toxicology 92, 39–51.

Antignac, J.P., Marchand, P., Gade, C., Matayron, G., Qannari, E.M., Le Bizec, B., et al., 2006. Studying variations in the PCDD/PCDF profile across various food products using multivariate statistical analysis. Anal. Bioanal. Chem. 384, 271–279.

Bandiera, S., Safe, S., Okey, A.B., 1982. Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene- or mixed-type inducers to cytosolic ah receptor. Chem. Biol. Interact. 39, 259–277.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci. 85, 2173–2179.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88, 2017–2026.

Beyer, A., Mackay, D., Matthies, M., Wania, F., Webster, E., 2000. Assessing long-range transport potential of persistent organic pollutants. Environ. Sci. Technol. 34, 699–703.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 7, 248–254.

Brambilla, G., Cherubini, G., De Filippis, S., Magliuolo, M., di Domenico, A., 2004. Review of aspects pertaining to food contamination by polychlorinated dibenzodioxins, dibenzofurans, and biphenyls at the farm level. Anal. Chim. Acta 514, 1–7.

Campbell, M.H., Miller, J.K., 1998. Effect of supplemental dietary vitamin E and zinc on reproductive performance of dairy cows and heifers fed excess iron. J. Dairy Sci. 81, 2693–2699.

Castillo, C., Hernández, J., Bravo, A., López Alonso, M., Pereira, V., Benedito, J.L., 2005. Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 169, 286–292.

Castillo, C., Hernández, J., Valverde, I., Pereira, V., Sotillo, J., López Alonso, M., Benedito, J.L., 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 80, 133–139.

Chamberland, G., Tremblay, A., Lamothe, P., Gignac, M., 1994. Clinical chemistry, growth and PCB levels in beef cattle exposed to a PCB fire. Toxicol. Environ. Chem. 44, 177–187.

Chen, G., Goeddel, D.V., 2002. TNF-R1 signaling: a beautiful pathway. Science 296, 1634–1635.

Cigliano, L., Strazzullo, M., Rossetti, C., Grazioli, G., Auriemma, G., Sarubbi, F., et al., 2014. Characterization of blood redox status of early and mid-late lactating dairy cows. Czech J. Anim. Sci. 59, 170–181.

Clark, G.C., Taylor, M.J., Tritscher, A.M., Lucier, G.W., 1991. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzop-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol. Appl. Pharmacol. 111, 422–431.

Dalton, T.P., Puga, A., Shertze, H.G., 2002. Induction of cellular oxidative stress by aryl hydrocarbon receptor activation. Chem. Biol. Interact. 141, 77–95.

Dutta, S.K., Mitra, P.S., Ghosh, S., Zang, S., Sonneborn, D., Hertz-Picciotto, I., et al., 2012. Differential gene expression and a functional analysis of PCB-exposed children: understanding disease and disorder development. Environ. Int. 40, 143–154.

European Union Reference Laboratory (EU-RL), 2009. Analytical capacities of National Reference Laboratories (NRLs) and Official Laboratories (OFLs) in case of dioxin incidents in the feed and food chain and conclusions for management in crisis situations. Community Reference Laboratory for Dioxins and PCBs in Feed and Food, pp. 1–35.

Ferrante, M.C., Amero, P., Santoro, A., Monnolo, A., Simeoli, R., Di Guida, F., et al., 2015. Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes. Toxicol. Appl. Pharmacol. 279, 401–408.

Fischer, L.J., Seegal, R.F., Ganey, P.E., Pessah, I.N., Kodavanti, P.R., 1998. Symposium overview: toxicity of non-coplanar PCBs. Toxicol. Sci. 41, 49–61.

Gasiewicz, T.A., Neal, R.A., 1979. 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol. Appl. Pharmacol. 51, 329–339.

Ghiselli, A., Serafini, M., Natella, F., Scaccini, C., 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic. Biol. Med. 29, 1106–1114.

Glynn, A., Aune, M., Nilsson, I., Darnerud, P., Bignert, A., Nordlander, I., 2009. Declining levels of PCB, HCB and p,p′-DDE in adipose-tissue from food producing bovines and swine in Sweden 1991–2004. Chemosphere 74, 1457–1462.

Guruge, K.S., Yamanaka, N., Hasegawa, J., Miyazaki, S., 2009. Differential induction of cytochrome P450 1A1 and 1B1 mRNA in primary cultured bovine hepatocytes treated with TCDD, PBDD/Fs and feed ingredients. Toxicol. Lett. 185, 193–196.

Haarmann-Stemmann, T., Bothe, H., Abel, J., 2009. Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways. Biochem. Pharmacol. 77, 508–520.

Halliwell, B., Gutteridge, J.M.C., 2000. Free radicals, other reactive species and disease. In: Halliwell, B., Gutteridge, J.M.C. (Eds.), Free Radicals in Biology and Medicine. Oxford University Press, Oxford, pp. 617–783.

Hassoun, E.A., Wang, H., Abushaban, A., Stohs, S.J., 2002. Induction of oxidative stress in the tissues of rats after chronic exposure to TCDD, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3′,4,4′,5-pentachlorobiphenyl. J. Toxicol. Environ. Health A 65, 825–842.

Huwe, J.K., Smith, D.J., 2005. Laboratory and on-farm studies on the bioaccumulation and elimination of dioxins from a contaminated mineral supplement fed to dairy cows. J. Agric. Food Chem. 53, 2362–2370.

Ishida, T., Takeda, T., Koga, T., Yahata, M., Ike, A., Kuramoto, C., et al., 2009. Attenuation of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity by resveratrol: a comparative study with different routes of administration. Biol. Pharm. Bull. 32, 876–881.

Kankofer, M., 2002. Placental release/retention in cows and its relation to peroxidative damage of macromolecules. Reprod. Domest. Anim. 37, 27–30.

Kankofer, M., Albera, E., Feldman, M., Gundling, N., Hoedemaker, M., 2010. Comparison of antioxidative/oxidative profiles in blood plasma of cows with and without retained fetal placental membranes. Theriogenology 74, 1385–1395.

Kim, M.J., Marchand, P., Henegar, C., Antignac, J.P., Alili, R., Poitou, C., Bouillot, J.L., Basdevant, A., Le Bizec, B., Barouki, R., Clément, K., 2011. Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. Environ. Health Perspect. 119, 377–383.

Kim, M.K., Oh, S., Lee, J.H., Im, H., Ryu, Y.M., Oh, E., et al., 2004. Evaluation of biological monitoring markers using genomic and proteomic analysis for automobile emission inspectors and waste incinerating workers exposed to polycyclic aromatic hydrocarbons or 2,3,7,8,-tetracholrodedibenzo-p-dioxins. Exp. Mol. Med. 36, 96–410.

Kliewer, S., 2003. The nuclear pregnane X receptor regulates xenobiotic detoxification. J. Nutr. 133, 2444S–2447S.

Lai, I., Chai, Y., Simmons, D., Luthe, G., Coleman, M.C., Spitz, D., et al., 2010. Acute toxicity of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) in male Sprague–Dawley rats: effects on hepatic oxidative stress, glutathione and metals status. Environ. Int. 36, 918–923.

Larsen, J.C., 2006. Risk assessments of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in food. Mol. Nutr. Food Res. 50, 885–896.

Lohman, K., Seigneur, C., 2001. Atmospheric fate and transport of dioxins: local impacts. Chemosphere 45, 161–171.

Lomba, F., 1996. Influence of dietary cation–anion and oxidative-antioxidants balances on diseases occurring around parturition in the dairy cows. Ann. Med. Vet. 140, 109–122.

Machala, M., Neča, A., Drábek, P., Ulrich, R., Šabatová, V., Nezveda, K., et al., 1998. Effects of chronic exposure to PCBs on cytochrome P450 systems and steroidogenesis in liver and testis of bulls (*Bos taurus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 120, 65–70.

Mandal, P.K., 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. J. Comp. Physiol. B. 175, 221–230.

Masahiko, N., Honkakoski, P., 2000. Induction of drug metabolism by nuclear receptor CAR: molecular mechanisms and implications for drug research. Eur. J. Pharm. Sci. 11, 259–264.

Matsumura, F., 2003. On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. Biochem. Pharmacol. 66, 527–540.

McLachlan, M.S., 1993. Mass balance of polychlorinated biphenyls and other organochlorine compounds in a lactating cow. J. Agric. Food Chem. 41, 474–480.

McLachlan, M.S., 1994. Model of the fate of hydrophobic contaminants in cows. Environ. Sci. Technol. 28, 2407–2414.

Miller, N.J., Rice-Evans, C.A., Davies, M.J., Gopinathan, V., Milner, A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. Clin. Sci. (Lond.) 84, 407–412.

Moos, A.B., Oughton, J.A., Kerkvliet, N.I., 1997. The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on tumor necrosis factor (TNF) production by peritoneal cells. Toxicol. Lett. 90, 145–153.

Nebert, D.W., Roe, A.L., Dieter, M.Z., Solis, W.A., Yang, Y., Dalton, T.P., 2000. Role of the aromatic hydrocarbon receptor and (Ah) gene battery in the oxidative stress response, cell cycle control, and apoptosis. Biochem. Pharmacol. 59, 65–85.

Olmedilla, B., Granado, F., Gil-Martinez, E., Blanco, I., Rojas-Hidalgo, E., 1997. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. Clin. Chem. 43 (6 Pt 1), 1066–1071.

Parkinson, A., Safe, S.H., Robertson, L.W., Thomas, P.E., Ryan, D.E., Reik, L.M., et al., 1983. Immunochemical quantitation of cytochrome p-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships. J. Biol. Chem. 258, 5967–5976.

Patterson Jr., D.G., Needham, L.L., Pirkle, J.L., Roberts, D.W., Bagby, J., Garrett, W.A., et al., 1988. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. Arch. Environ. Contam. Toxicol. 17, 139–143.

Pelclova, D., Navratil, T., Fenclova, Z., Vlckova, S., Kupka, K., Urban, P., 2011. Increased oxidative/nitrosative stress markers measured non-invasively in patients with high 2,3,7,8-tetrachloro-dibenzo-p-dioxin plasma level. Neuro Endocrinol. Lett. 32 (Suppl 1), 71–76.

Quaye, I.K., 2008. Haptoglobin, inflammation and disease. Trans. R. Soc. Trop. Med. Hyg. 102, 735–742.

Rossi, F., Bertuzzi, T., Vitali, A., Rubini, A., Masoero, F., Morlacchini, M., Piva, G., 2010. Monitoring of the declining trend of polychlorobifenyls concentration in milk of contaminated dairy cows. Ital. J. Anim. Sci. 9, 88–92.

Rychen, G., Ducoulombier, C., Grova, N., Jurjanz, S., Feidt, C., 2005. Terms and risk of transfer of persistent organic pollutants into milk. Inra Prod. Anim. 18, 355–366.

Rychen, G., Jurjanz, S., Fournier, A., Toussaint, H., Feidt, C., 2014. Exposure of ruminants to persistent organic pollutants and potential of decontamination. Environ. Sci. Pollut. Res. Int. 21, 6440–6447.

Saeed, S.A., Ahmad, N., Ahmed, S., 2007. Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: its polymorphism and relation to hemoglobin binding. Biochem. Biophys. Res. Commun. 353, 915–920.

Safe, S.H., 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. 26, 371–399.

Schecter, A., Birnbaum, L., Ryan, J.J., Constable, J.D., 2006. Dioxins: an overview. Environ. Res. 101, 419–428.

Selgrade, L.K., 2007. Immunotoxicity: the risk is real. Toxicol. Sci. 100, 328–332.

Senft, A.P., Dalton, T.P., Nebert, D.W., Genter, M.B., Hutchinson, R.J., Shertzer, H.G., 2002. Dioxin increases reactive oxygen production in mouse liver mitochondria. Toxicol. Appl. Pharmacol. 178, 15–21.

Shertzer, H.G., Nebert, D.W., Puga, A., Ary, M., Sonntag, D., Dixon, K., et al., 1998. Dioxin causes a sustained oxidative stress response in the mouse. Biochem. Biophys. Res. Commun. 253, 44–48.

Shertzer, H.G., Vasiliou, V., Liu, R.M., Tabor, M.W., Nebert, D.W., 1995. Enzyme induction by L-buthionine (S,R)-sulfoximine in cultured mouse hepatoma cells. Chem. Res. Toxicol. 8, 431–436.

Slezak, B.P., Hamm, J.T., Reyna, J., Hurst, C.H., Birnbaum, L.S., 2002. TCDD-mediated oxidative stress in male rat pups following perinatal exposure. J. Biochem. Mol. Toxicol. 16, 49–52.

Slezak, B.P., Hatch, G.E., DeVito, M.J., Diliberto, J.J., Slade, R., Crissman, K., et al., 2000. Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Sci. 54, 390–398.

Sordillo, L.M., Aitken, S.L., 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol. 128, 104–109.

Spagnuolo, M.S., Cigliano, L., Balestrieri, M., Porta, A., Abrescia, P., 2001. Synthesis of ascorbate and urate in the ovary of water buffalo. Free Radic. Res. 35, 233–243.

Spagnuolo, M.S., Cigliano, L., Nebbia, C., Rossetti, C., Grazioli, G., Iannuzzi, L., 2012. Analysis of plasma indices of redox homeostasis in dairy cows reared in polluted areas of Piedmont (northern Italy). Sci. Total Environ. 433, 450–455.

Spagnuolo, M.S., Maresca, B., La Marca, V., Carrizzo, A., Veronesi, C., Cupidi, C., et al., 2014. Haptoglobin interacts with apolipoprotein E and beta-amyloid and influences their crosstalk. ACS Chem. Neurosci. 5, 837–847.

Spagnuolo, M.S., Sarubbi, F., Rossetti, C., Grazioli, G., Di Meo, G.P., Iannuzzi, L., 2011. Effect of dioxin exposure on several indices of blood redox status in lactating buffalo cows. J. Dairy Res. 4, 1–6.

Thomas, G.O., Jones, J.L., Jones, K.C., 2002. Polychlorinated dibenzo-pdioxin and furan (PCDD/F) uptake by pasture. Environ. Sci. Technol. 36, 2372–2378.

Thomas, G.O., Sweetman, A.J., Jones, K.C., 1999a. Metabolism and body-burden of PCBs in lactating dairy cows. Chemosphere 39, 1533–1544.

Thomas, G.O., Sweetman, A.J., Jones, K.C., 1999b. Input–output balance of polychlorinated biphenyls in a long-term study of lactating dairy cows. Environ. Sci. Technol. 33, 104–112.

Uchida, K., Stadtman, E.R., 1993. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. J. Biol. Chem. 268, 6388–6393.

United Nations Environmental Program (UNEP), 2001. Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants (Geneva) .

Wajant, H., Pfizenmaier, K., Scheurich, P., 2003. Tumor necrosis factor signaling. Cell Death Differ. 10, 45–65.

Wathes, D.C., Clempson, A.M., Pollott, G.E., 2012. Associations between lipid metabolism and fertility in the dairy cow. Reprod. Fertil. Dev. 25, 48–61.

Waxman, D., 1999. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch. Biochem. Biophys. 369, 11–23.

Wens, B., De Boever, P., Maes, M., Hollanders, K., Schoeters, G., 2011. Transcriptomics identifies differences between ultrapure non-dioxin-like polychlorinated biphenyls (PCBs) and dioxin-like PCB126 in cultured peripheral blood mononuclear cells. Toxicology 287, 113–123.

Westerink, R.H., 2014. Modulation of cell viability, oxidative stress, calcium homeostasis, and voltage- and ligand-gated ion channels as common mechanisms of action of (mixtures of) non-dioxin-like polychlorinated biphenyls and polybrominated diphenyl ethers. Environ. Sci. Pollut. Res. Int. 21, 6373–6383.

Whitlock, J.P.J., 1990. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. Annu. Rev. Pharmacol. Toxicol. 30, 251–277.

Wilde, D., 2006. Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. Anim. Reprod. Sci. 96, 240–249.

Zhao, W., Ramos, K.S., 1998. Modulation of hepatocyte gene expression by the carcinogen benzo[a]pyrene. Toxicol. in Vitro 12, 175–182.