Reproductive Toxicology 101 (2021) 33–49

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Role of aryl hydrocarbon receptor (AHR) in overall retinoid metabolism: Response comparisons to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure between wild-type and AHR knockout mice

Javier Esteban [a,1], Ismael Sánchez-Pérez [a,1], Gerd Hamscher [b], Hanna M. Miettinen [c], Merja Korkalainen [d], Matti Viluksela [c,d], Raimo Pohjanvirta [e,*,2], Helen Håkansson [f,2]

[a] Instituto De Bioingeniería, Universidad Miguel Hernández De Elche, Elche, Alicante, Spain
[b] Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Giessen, Germany
[c] School of Pharmacy (Toxicology) and Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland
[d] Environmental Health Unit, Finnish Insitute for Health and Welfare (THL), Kuopio, Finland
[e] Department of Food Hygiene & Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Mustialankatu 1, FI-00790 Helsinki, Finland
[f] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Keywords:
Aryl hydrocarbon receptor
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDD
Genetically modified organisms
Retinoids
Vitamin A

ABSTRACT

Young adult wild-type and aryl hydrocarbon receptor knockout (AHRKO) mice of both sexes and the C57BL/6J background were exposed to 10 weekly oral doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; total dose of 200 μg/kg bw) to further characterize the observed impacts of AHR as well as TCDD on the retinoid system. Unexposed AHRKO mice harboured heavier kidneys, lighter livers and lower serum all-*trans* retinoic acid (ATRA) and retinol (REOH) concentrations than wild-type mice. Results from the present study also point to a role for the murine AHR in the control of circulating REOH and ATRA concentrations. In wild-type mice, TCDD elevated liver weight and reduced thymus weight, and drastically reduced the hepatic concentrations of 9-*cis*-4-oxo-13,14-dihydro-retinoic acid (CORA) and retinyl palmitate (REPA). In female wild-type mice, TCDD increased the hepatic concentration of ATRA as well as the renal and circulating REOH concentrations. Renal CORA concentrations were substantially diminished in wild-type male mice exclusively following TCDD-exposure, with a similar tendency in serum. In contrast, TCDD did not affect any of these toxicity or retinoid system parameters in AHRKO mice. Finally, a distinct sex difference occurred in kidney concentrations of all the analysed retinoid forms. Together, these results strengthen the evidence of a mandatory role of AHR in TCDD-induced retinoid disruption, and suggest that the previously reported accumulation of several retinoid forms in the liver of AHRKO mice is a line-specific phenomenon. Our data further support participation of AHR in the control of liver and kidney development in mice.

Abbreviations: AHR, aryl hydrocarbon receptor; AHRKO, AHR knockout; ANOVA, analysis of variance; ATRA, all-*trans* retinoic acid; CAR, constitutive androstane receptor; CM, chylomicrons; CORA, 9-*cis*-4-oxo-1314-dihydroretinoic acid; CRABP, cellular retinoic acid-binding protein; CRABP2, cellular retinoic acid-binding protein type 2; CRBP, cellular retinol-binding protein; CRBP1, cellular retinol-binding protein type 1; CYP, cytochrome P450; EATS, estrogen, androgen, thyroid hormone, and steroidogenesis; FXR, farnesoid X receptor; HPLC, high-performance liquid chromatography; IA, interaction; LOD, limit of detection; LRAT, lecithin: retinol acetyltransferase; LXR, liver X receptor; NS, not significant; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAL, retinal; RALDH, retinal dehydrogenase; RAR, retinoic acid receptor; RARE, retinoic acid response element; RBP, retinol-binding protein; RE, retinyl ester; REOH, retinol; REPA, retinyl palmitate; RXR, retinoid X receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEF, toxic equivalency factor; TEQ, toxic equivalency; TR, thyroid hormone receptor; VDR, vitamin D receptor; WHO, World Health Organization; WT, wildtype.

* Corresponding author.
E-mail addresses: jesteban@umh.es (J. Esteban), ismael.sanchez@goumh.umh.es (I. Sánchez-Pérez), Gerd.Hamscher@lcb.Chemie.uni-giessen.de (G. Hamscher), hanna.m.miettinen@uef.fi (H.M. Miettinen), merja.korkalainen@thl.fi (M. Korkalainen), matti.viluksela@uef.fi (M. Viluksela), raimo.pohjanvirta@helsinki.fi (R. Pohjanvirta), helen.hakansson@ki.se (H. Håkansson).
[1] Equal contribution first author.
[2] Equal contribution last author.

https://doi.org/10.1016/j.reprotox.2021.02.004
Received 14 October 2020; Received in revised form 20 January 2021; Accepted 10 February 2021
Available online 16 February 2021

1. Introduction

1.1. Preface

Despite several lines of converging evidence and scientific regulatory discussions linking AHR biology and TCDD-induced toxicity to a retinoid system-associated mode of action, there are few published studies on the retinoid system in AHRKO mouse models [1–4]. Here, we report of an experimental study designed to determine the influence of AHR over- and inactivation on the overall retinoid metabolism by analysing concentrations of a selected profile of endogenous retinoid compounds in liver, kidneys, and circulation of adult male and female mice using the AHRKO line developed in Prof. Chris Bradfield's laboratory [5]. The "overall retinoid metabolism" covered in the current study is limited to concentration analyses of a small number of retinoid forms in selected organs and circulation. These analysed retinoid compounds reflect the metabolic steps regulating intracellular storage and release of retinyl esters, as well as intracellular synthesis and degradation of retinoic acids. Liver, kidneys, and serum were chosen for retinoid analyses in this study, as together these tissues are crucial in the overall whole-body absorption, metabolism, distribution, and elimination of the dietary-derived vitamin A. In many instances, and for the purpose of this study, this selection can be seen as representative for the retinoid system in most organisms since most, if not all, cells carry complete machineries to fulfil fundamental physiological roles played both by AHR and the retinoid system for health in general over the life-course. TCDD, in turn, may adversely affect these life processes in many, if not all, cells and organs. An important intention of this study is to support ongoing regulatory initiatives dedicated to science-based incorporation of the retinoid system into test programs for chemical safety evaluations in the many different domains of human and wildlife health as recently reviewed [6,7,169]. To this end, the obtained original retinoid data from the experimental part of this study were further evaluated in relation to previously published data and by the use of mode-of-action and weight-of-evidence types of analytical approaches to derive additional regulatory-relevant insights. The experimental study background, design, and results are therefore embedded and evaluated in comprehensive, yet narrative, and review-style Introduction and Discussion sections of this article, with the aim to provide, in parallel, a broader context of both AHR and retinoid biology as a common background to the interpretation and conclusions of the presented original data.

1.2. Physiological roles of the AHR

The aryl hydrocarbon receptor (AHR) is an evolutionarily conserved over 600-million-year-old transcription factor, which is activated or repressed upon binding small endogenous or exogenous molecules [8]. It was discovered as a result of mechanistic studies to clarify the mode-of-action of dioxins[3] [9,10] and related environmental pollutants among the aryl hydrocarbons. It is now well known that AHR is expressed from the earliest stages of life in virtually all vertebrate cells, and concentrations of the receptor varies widely among cell types, tissues, and life stages [11,12]. Generation and characterization of AHR knockout (AHRKO) models have been and continue to be important in unveiling and establishing fundamental roles of AHR in physiology and pathology. Initial characterizations of AHRKO mouse lines demonstrated that AHR plays important roles in growth, organ development, and endocrine and metabolic homeostasis over the life-course (reviewed by [11,13–15]). Decreased or slower perinatal growth was observed in all three mouse lines initially created [5,16–18], while survival, fertility, immunology parameters, as well as liver size development, pathology and biochemistry showed similarities but also differences among the three lines in these initial studies [19]. Studies in older AHRKO mice revealed cardiac hypertrophy and hypertension along with elevated HIF-1α protein expression in the absence of hypoxia [20–22,40]. In addition to this cardiac phenotype, AHRKO mice exhibited a premature ageing process accompanied by pathological lesions in multiple organs such as the liver (portal vascular hypertrophy, hepatocellular tumors), gastrointestinal tract (pyloric hypertrophy, rectal prolapse), uterus (hypertrophy, thromboses and mineralization of serosal vessels), spleen (T and/or B cell depletion), and skin (alopecia and ulcers) [20]. Additional mouse phenotyping studies have revealed key roles of AHR in fibrosis [14,21], haematopoiesis [5,23], inflammatory processes [24,25], and reproduction, including proper pregnancy, teratogenicity, and fetal survival [26,27], normal ovarian germ cell dynamics [28–30], and functioning of seminal vesicles and the coagulation gland [31], while its role in normal testicular development is controversial [32,33]. Detailed investigations in one AHRKO mouse line have revealed that AHR is required for resolution of fetal vascular structures in the liver (ductus venosus), eyes (hyaloid artery), and kidneys (fetal-like vascular architecture) [34]; it may further be necessary for regeneration of adult tissues, such as liver and lung [35,36]. Recent follow-up of the premature ageing phenotype revealed, in addition, that lack of AHR shortens the life-span and has an impact on behaviour as well as anatomical brain structures (enhanced astrogliosis in hippocampus and loss of white matter integrity) [24]. A finding that appears to be directly relevant to humans [37] is congenital nystagmus associated with an altered optic nerve myelin sheath and inflammatory gene expression in AHRKO mice [38,39].

Neither the markedly reduced liver size nor the enlarged heart phenotype that are observed in AHRKO mice are duplicated in the AHRKO rat; instead the AHRKO rat has a pronounced urinary tract phenotype, consisting of hydronephrosis and -ureter [40]. In a comparative study by Harrill et al. [40], both AHRKO rats and mice displayed enlarged kidneys, although in mice there was no associated pathology. In a different AHRKO mouse line, however, higher amounts of glomerular and interstitial fibrosis were observed in kidneys compared with the corresponding wildtype [21]. In addition to mouse line and rodent species variations in response to AHR ablation, there are some reported sex differences in *e.g.*, serum clinical chemistry measurements and organ weights [40]. Studies in AHRKO models have overall revealed that experimental environment, genetic background, gene KO targeting strategies, sex, and species are factors which contribute to the precise physiological responses to AHR ablation reported so far, suggesting that the consequences of interference with the endogenous regulation of the AHR pathway are context-dependent. Taken together, phenotypic findings observed in mammalian AHRKO models convincingly demonstrate that AHR plays key roles in fetal growth and organ development and, throughout life, acts as a mediator of cellular homeostasis, including both metabolic and endocrine regulation.

1.3. AHR as a mediator of TCDD toxicity

In addition to the role of AHR in normal development and in postnatal physiology, there is also an important role of AHR as a mediator of cell defence and adaptation mechanisms in response to exposure to environmental toxicants. The best-studied and most potent exogenous

[3] The term dioxin includes the polychlorinated dibenzo-*p*-dioxins and dibenzofurans, and the dioxin-like polychlorinated biphenyls. These ubiquitious food contaminants also belong to the family of chemicals known as persistent organic pollutants (POPs), which are destined to international phase-out under the Stockholm convention [165] due to their toxicological properties in combination with their high physico-chemical and biological stability in the environment and in living organisms.

activator of AHR is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),[4] and numerous studies have established that exposure to TCDD causes a sustained over-activation of AHR in a large number of animal and cell models even at low environmentally relevant exposure levels. The toxicological manifestations following TCDD exposure depend on species and include a wasting syndrome associated with failure in body weight regulation, keratinization of epithelial linings and the skin condition chloracne, thymic atrophy and immunosuppression, hepatotoxicity, carcinogenesis, as well as reproductive, teratogenic and developmental toxicity, which is manifested in many organs and include behavioural deficits (reviewed in [10,41–46]). The reviewed toxicological studies have also revealed numerous clinical, biochemical, and endocrine end-points in response to TCDD exposure, including effects on various retinoid forms found in organs, cells and circulation of many species and strains.

The mandatory role of AHR in TCDD toxicity has been demonstrated for a wide range of these observed pathological and biochemical manifestations [1–4,17,33,40,47,48]. Similar to the AHR deficiency signs, also the signs and features of AHR over-activation by TCDD suggest that an endogenous overall whole-body and cell regulatory system is affected. First, depending on the dose of TCDD, duration of exposure, and life-stage when exposure starts, outcomes may span from mildly beneficial to severely detrimental. Next, TCDD lethality is markedly delayed (2–6 weeks after a single high dose), supporting a global, rather than a specific and local, type of toxicity. Likewise, the striking lack of cytotoxicity response to TCDD exposure in most cell lines, despite the dose-related and often pronounced biochemical response(s) to AHR over-activation in these cell lines, also suggests that a physiological cell context is required for the toxicity to manifest. In this regard, it has been demonstrated that AHR activation and signaling requires communication among different cell types in order to convey its functions. For example, closure of ductus venosus and the associated small liver size phenotype require appropriate AHR activation and signalling both in endothelial and haematopoietic cells [175], while the cleft palate phenotype in mice requires appropriate activation and signalling both in nasal epithelium and mesenchyme [49]. Furthermore, the metabolic and inflammatory responses to TCDD exposure of the liver are dependent on AHR activation in the hepatocytes alone [50], while additional AHR activation in the non-parenchymal stellate cells seems to be needed for the full liver toxicity to manifest (reviewed by [14]).

The most extensively examined AHR-regulated genes activated by TCDD encode the AHR battery of xenobiotic-metabolizing enzymes, inclusive of the cytochrome P450 (CYP) enzymes CYP1A1, CYP1A2, and CYP1B1, along with several Phase II enzymes. In addition, TCDD induces or represses a wide variety of other genes [170], but the connections between TCDD toxicity and gene expression changes are in need of much further investigation.

1.4. Crosstalk between AHR and retinoids

Apart from TCDD and related environmental toxicants, a large number of dietary, pharmaceutical, microbial and endogenous compounds can influence AHR activity. These include retinoic acid[5], together with its metabolic and transcriptional machinery [49,51]. Using a medaka fish embryo model, Hayashida et al. [51] provided morphological and gene expression evidence that retinoic acid and its receptors, *i.e.* RARs and RXRs, are required for the expression of AHR mRNA, and thus for AHR-regulated gene transcription. The authors proposed a feed-back mechanism regulating *in vivo* retinoic acid-levels, in which excessive synthesis of retinoic acid activates AHR mRNA expression and then, in turn, increased activity of AHR stimulates conversion of retinoic acid to inactive metabolites [51]. Likewise, Jacobs et al. [49], who used a genetic approach in the mouse embryo to study palate development, demonstrated that the AHR transcript level in the nasal mesenchyme is controlled through a retinal dehydrogenase 3-generated retinoic acid signal in the nasal epithelium, which is transduced by RARγ activation. However, these authors ruled out that retinoic acid would bind to and activate AHR, as a ligand, to produce cleft palate [49]. Together, these studies show that AHR expression is controlled by retinoic acid-activated RAR in several different developing tissue structures. Furthermore, these data indicate that these two cellular regulators work together to control growth, as well as organ development and function.

1.5. Brief overview of the retinoid system

As indicated in Fig. 1, the retinoid system as a whole is represented by the intake and absorption of different dietary forms of vitamin A[6], and the *in situ* synthesis, distribution, and use of a variety of endogenous retinoid forms, among which the simple and small all-*trans*-retinoic acid (ATRA) molecule, is acting in a remarkably complex manner over the life-course, and furthermore, is strictly controlled on spatio-temporal scales and on cellular and whole organism levels. Chemically more complex transcriptionally active and endogenously formed retinoic acid molecules as compared with ATRA have been described during the last decades [52–55,161], while the apolar retinoid forms retinol (REOH) and retinyl esters (REs) were identified during the 1930s (reviewed in [56]) (Fig. 2). Processes controlled by retinoic acid(s) include morphogenesis, transcriptional regulation, epigenetics, as well as extensive hormonal cross-talk with metabolic and endocrine sensors within the large and transcriptionally active family of nuclear receptors (reviewed in [57–61]). In contrast to the classical hormones, which are synthesized from endogenous precursors in response to physiological cues, retinoic acid isomers and metabolites are derived from a vital nutrient, which is equipped with a complex metabolic machinery operating on cell, organ, and whole-body levels (Fig. 1). The embryo is dependent on maternal supplies of these retinoic acids, and from birth onwards the organism is dependent on their dietary intake to continuously cope with times of deficiency as well as with excessive intakes. These fundamental features of the retinoid system in life-processes are well in line with the evolutionary conservation of the RARs and RXRs [62–64,174], which in turn is remarkably comparable to the evolutionary conservation of AHR [8].

1.6. Role of retinoids in TCDD toxicity

The reported pathological changes following AHR over-activation by TCDD show striking resemblance with deficiency or excess conditions of vitamin A in many species and strains [65–68,69,83]. These similarities include disturbances in body weight regulation, coordination of cell differentiation processes, fertility and spermatogenesis. It has also been shown that retinoids play important roles in the pathophysiology of several hepatic diseases, including fatty liver, portal fibrosis, chirrosis and hepatocellular carcinoma [70–72], which are compatible with reports of dioxin-induced liver toxicity [73,74], as well as the adult liver phenotype of AHRKO mice (reviewed by [14]). Likewise, TCDD-induced bone lesions and developmental malformations, such as cleft palate

[4] TCDD is the reference compound for the regulated group of dioxin-like molecules, and is assigned a relative potency factor of 1 in the regulatory toxic equivalency (TEQ)-tool, which has been developed to assess the health impact of dietary exposures to mixtures of dioxins [86,87].

[5] Retinoic acid is endogenously synthesized from dietary sources of vitamin A and occurs in several forms *in vivo*, including all-*trans* and 13-*cis* retinoic acid and associated metabolites.

[6] Vitamin A is essential both for normal embryofetal development and for maintenance of homeostasis over the life-course [166]. Vitamin A-active substances are defined as compounds which exhibit qualitatively the biological activity of REOH. The term retinoid on the other hand, includes both the natural and synthetic analogues of REOH with or without biological activity [167].

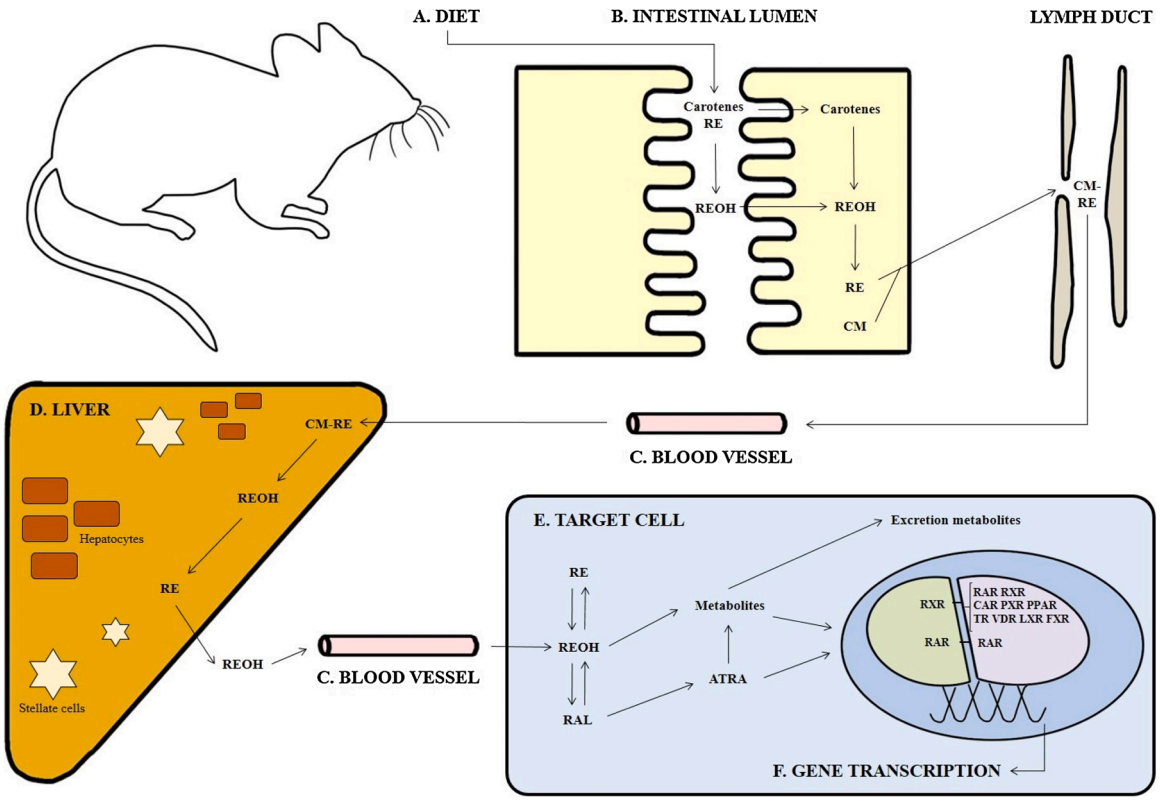

Fig. 1. Schematic presentation of the overall retinoid system (adapted from [58]). Briefly, dietary vitamin A (A) in the form of carotenes, retinol (REOH), or retinyl esters (REs) undergoes gastrointestinal absorption and transformation into the physiologically formed REs (B). *Via* lymph and blood circulation (C), and bound to chylomicrons (CM), the REs are internalized in the liver (D), where they are stored as such in lipid droplets of hepatocytes or in stellate cells, or they are released as REOH into the blood circulation (C) for distribution to extrahepatic organs, including the adipose tissue, which harbors 15–20 % of the retinoids in the body [168]. Local metabolism in target cells of virtually all organs and cell types, including the liver (E), facilitates the synthesis of additional functional retinoid forms, such as retinal (RAL), all-*trans*-retinoic acid (ATRA), and the recently described metabolites 9-*cis*-4-oxo-13,14-dihydroretinoic acid (CORA) and 9-*cis*-13,14-dihydroretinoic acid (Fig. 2), as well as their degradation products. As indicated in the target cell compartment of the figure (E), retinoids have important functions in many biological processes, perhaps most notably in gene transcription (F), which is mediated *via* the retinoic acid receptor families RARs and RXRs, of which the latter is an obligate heterodimer partner of the xenobiotic-, nutrient-, and hormone-sensing receptors CAR, PXR, LXR, FXR, PPAR, VDR, and TR. Retinoids have a central role in the patterning and specification of cells in most organ systems during embryogenesis and fetal development. In adult life, retinoids support growth, vision, maintenance of numerous epithelial tissues, reproduction and overall survival. The aldehyde form, RAL, is required for proper vision, while carefully regulated levels of retinoic acid, acting *via* RARs and RXRs, fulfill transcriptional functions. Multiple forms of the RARs/RXRs and their subtypes, response elements on the genome, and binding proteins (RBP, CRBP and CRABP) together with the large number of enzymes that are involved in retinoid metabolism, add to the complexity. For abbreviations used in the figure, see the Abbreviation list.

formation, bear similarities with signs and pathologies of vitamin A excess (reviewed in [49,51,67]).

1.7. Aims of the current study

The study aims were to determine the influence of AHR over- and inactivation on a profile of endogenous retinoid analytes in representative organs of adult male and female mice, and to contrast the measured retinoid concentrations with previously published data on them. An additional aim was to evaluate how the results of the present study are compatible with the established AHR mode-of-action, and with published data on AHR-mediated TCDD toxicity.

2. Materials and methods

2.1. Chemicals

TCDD (UFA Oil Institute, Ufa, Russia) was 99 % pure and dissolved in corn oil (Sigma Chemicals, St. Louis, MO). The acids, ATRA, 9-*cis*retinoic, 13-*cis*retinoic, and also the internal standards, acitretin and retinyl acetate, were provided by Sigma-Aldrich Química (Madrid, Spain). The all-*trans*retinoic acid metabolites, 13-*cis*-4-oxoretinoic acid, 13-*cis*-4-OH-retinoic acid, and CORA were kindly provided by the formerly named Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine, Hannover, Germany. Ammonium acetate was supplied by Panreac (Barcelona, Spain), chloroform, ethanol, isopropanol and methanol (HPLC grade) by J.T. Baker. Other reagents were of analytical purity and supplied by local suppliers. Deionized water was purified by a Milli Q unit (Millipore, Molsheim, France). All solvents were HPLC grade at least, and obtained from Merck (Darmstadt, Germany) or Mallinckrodt Baker (Greisheim, Germany).

2.2. Animals

Wild-type and AHRKO mice in a C57BL/6J background (originally generated in Dr. Chris Bradfield's lab [5]) were obtained from The Jackson Laboratory (Bar Harbor, ME; USA) and maintained using heterozygous breeding. They were kept in a conventional laboratory animal unit subjected regularly to health surveys consisting of serological and bacteriological screening as suggested by FELASA [75]. These surveys indicated that the animals were free of typical rodent pathogens. The wild-type and AHRKO mice used in the present study originated from the same litters and were identified by PCR from auricular punches. The mice were acclimated to the experimental conditions for one week

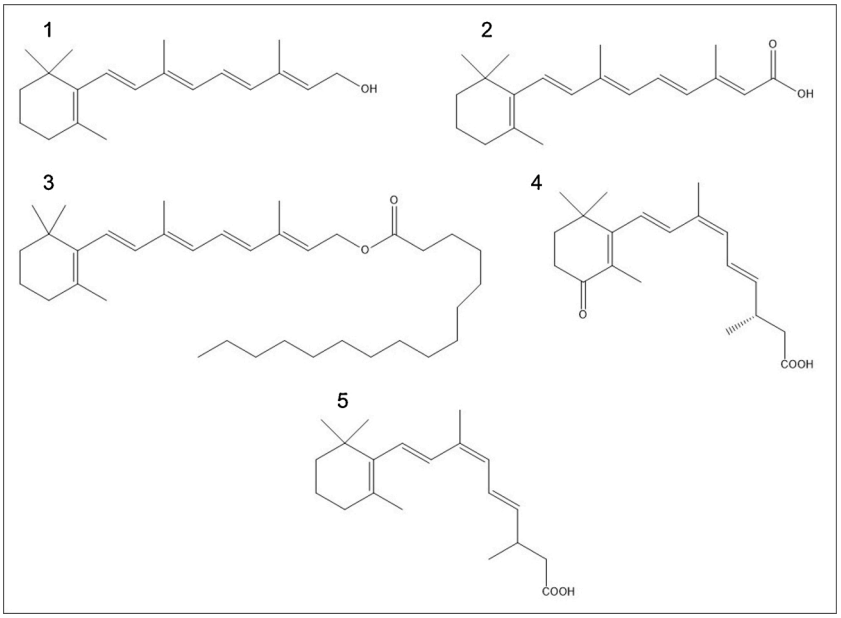

Fig. 2. Structural formulae of some retinoid species *i.e.* 1) retinol (REOH), 2) all-*trans* retinoic acid (ATRA), 3) retinyl palmitate (REPA), 4) 9-*cis*-4-oxo-13,14-dihydroretinoic acid (CORA), and 5) 9-*cis*-13,14-dihydroretinoic acid.

before commencing with dosing. At the start of the treatment, the mice were 8–12 weeks old, and randomized by body weight into treatment groups of 6 males and 6 females per genotype. The mice were housed individually in stainless steel wire-bottomed cages and given standard pelleted R36 feed (Lactamin, Stockholm, Sweden; contains vitamin A 12,000 IU/kg) and tap water *ad libitum*. The room was artificially illuminated from 7 a.m. to 7 p.m., and air-conditioned to provide about 8 air changes per hour. The ambient temperature was 21 ± 1 °C and the relative humidity 50 ± 10 %. The animals were individually identified by notching of pinnae, and the treatment groups were labeled with color codes.

2.3. Ethical permit and experimental design

The study protocol was approved by the Animal Experiment Committee of the University of Kuopio (license No. 05-42) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The study was carried out as described by Herlin et al. [76]. Solutions of TCDD were mixed and ultrasonicated for 20 min before administration by oral gavage at 4 ml/kg. Control mice were given pure corn oil. To rapidly achieve the kinetic steady state, the total dose of TCDD (200 μg/kg) was divided into one loading dose (40 μg/kg) and 9 maintenance doses (18 μg/kg), in weekly intervals (τ), which were calculated according to the formula [77]:

$$x_0^* = x_0\left(\frac{1}{1-e^{-K\tau}}\right)$$

where x_0^* = loading dose

x_0 = maintenance dose

K = elimination rate constant $\left(=\frac{\ln 2}{t_{½}}\right)$

τ = dosing interval = 7 days

$t½$ = half-life = 8 days in wild-type C57BL/6J mice [78]

At the end of the treatment period of 10 weeks (one week after the last maintenance dose), the mice were anesthetized with CO_2/O_2 (70/30 %). Blood samples were drawn from the left ventricle using Venoject needles (Terumo) and blood collection tubes, and the mice were killed by exsanguination. Liver, kidney, thymus, epididymis and testis were weighed, liver and kidney were snap frozen in liquid nitrogen and stored at −80 °C for retinoid analysis.

2.4. Retinoid analysis

Retinoids were extracted from frozen samples of liver, kidneys, and serum as previously described [79,80]. ATRA and CORA were extracted from tissues and were analyzed as reported by Schmidt et al. [54]. Briefly, retinoids were extracted with isopropanol from tissue homogenate or serum. Separation of ATRA, CORA, REOH and REPA was achieved by solid-phase-extraction using an aminopropyl-phase. ATRA and CORA were separated on a Spherisorb ODS2 column (Waters, Eschborn, Germany) using a binary HPLC (Shimadzu, Duisburg, Germany) gradient, and were detected with an UV detector at 340 nm. REOH and REPA were separated on a J'sphere ODS-H80 column (YMC Schermbeck, Germany) using a binary gradient, and detected at 325 nm. Limits of detection (LODs) for ATRA and CORA were 0.6 and 1.0 pmol/g liver, respectively. LODs for REOH and REPA were 5.6 and 5.5 pmol/g liver, respectively.

2.5. Statistical analysis

The data are provided as mean ± SD. Three-way analysis of variance (ANOVA) with sex, genotype and treatment as fixed factors was used for the overall statistical assessment of the data. The normality of data distribution was analysed by Shapiro-Wilk's test for each cell of the design, outliers were detected by boxplots, and variance homogeneity was evaluated by Levene's test. In the case of skewed data distribution, extreme outliers, or non-homogeneous variances, log10 and square root transformations were attempted. If these failed to rectify the issue, the data were analyzed both with and without the outlier(s) and, occasionally, also after conversion of the outlier to a less extreme value retaining the original rank. Simple main effects were assessed using the overall error term. Statistics on the three-way and two-way interactions are provided for each variable analysed in Figs. 2–6 and Supplementary Figs. S1–S4. Moreover, one-way ANOVA was performed separately on male and female data broken down by genotype and treatment followed by the Student-Newman-Keuls post-hoc test for pairwise comparisons if the variances were homogenous, or by Welch's ANOVA followed by Games-Howell multiple comparisons if they were not. For skewed data

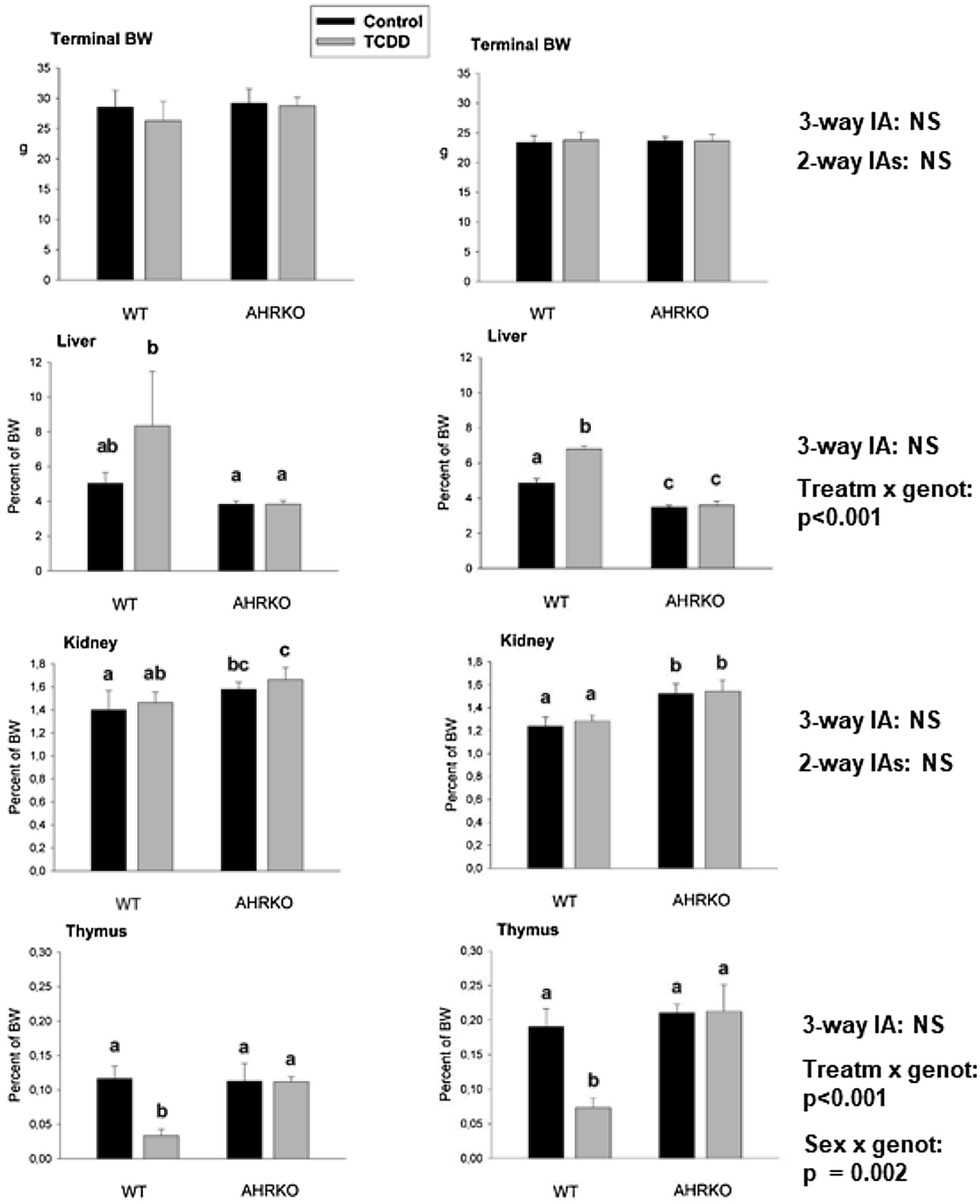

Fig. 3. Effects of TCDD on body weight at termination and the relative (% of body weight) weights of the liver, kidneys and thymus in male (left panels) and female wild-type (WT) & AHRKO mice. Group mean + SD, n = 6. Columns with non-identical letters are statistically different at the significance level of $p < 0.05$. IA, interaction; NS, not significant.

or extreme outliers, data transformation was first attempted as above for three-way ANOVA. If this was unsuccessful, the Kruskal-Wallis nonparametric ANOVA was employed followed by pair-wise multiple comparisons adjusted by the Bonferroni correction. The outcomes of these parametric and nonparametric one-way ANOVAs are also shown in Figs. 2–6 and Supplementary Figs. S1–S4. Finally, for direct comparisons of the two sexes or genotypes, control groups were contrasted by the *t*-test or Welch's *t*-test depending on variance homogeneity. Data distribution abnormalities and outliers were treated as above. The level of statistical significance was always set at $p<0.05$.

2.6. Literature review

A small narrative literature study, covering all published original

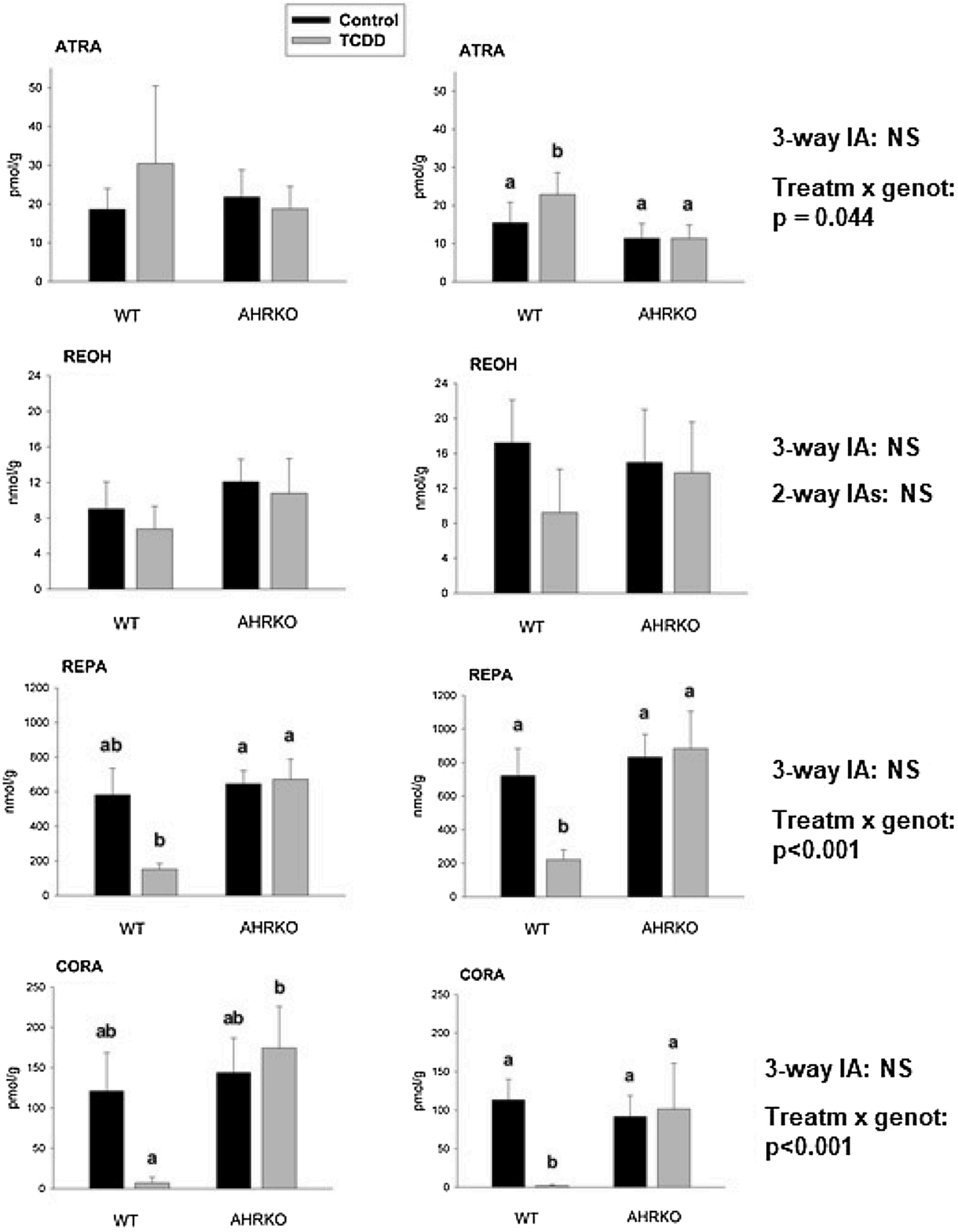

Fig. 4. Effects of TCDD on hepatic concentrations of ATRA, REOH, REPA and CORA in male (left panels) and female wild-type (WT) & AHRKO mice. Group mean + SD; n = 4–6. Columns with non-identical letters are statistically different at the significance level of $p < 0.05$. IA, interaction; NS, not significant.

studies where tissue retinoid concentrations were reported in mammalian AHRKO models, was performed. Retrieved data were tabulated together with detailed study design information of importance for regulatory interpretations and assessments of scientific data.

3. Results

3.1. Body and organ weights

At the onset, male mice were significantly ($p < 0.001$) heavier than females. Although females grew faster ($p = 0.018$ for body weight

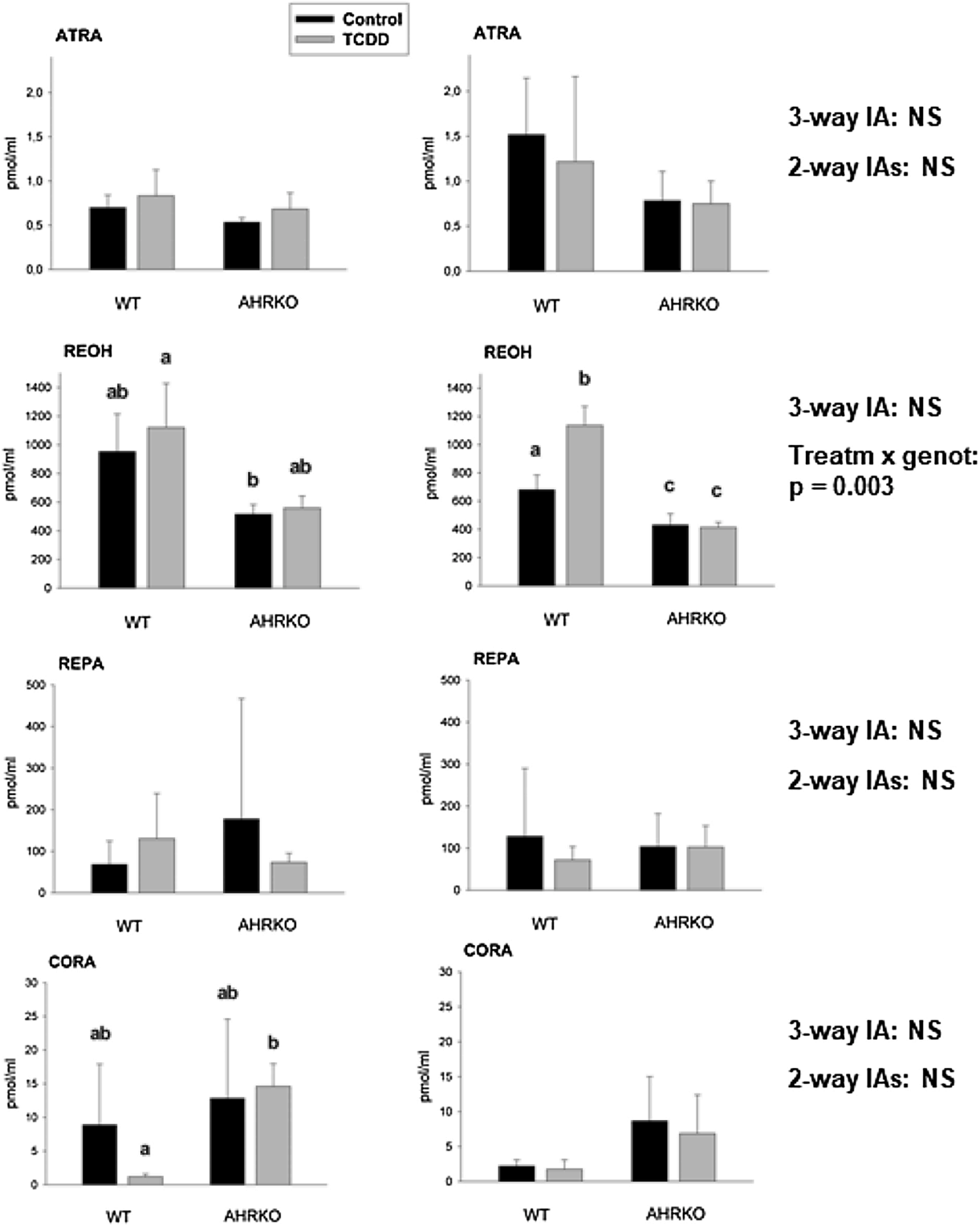

Fig. 5. Effects of TCDD on serum concentrations of ATRA, REOH, REPA and CORA in male (left panels) and female wild-type (WT) & AHRKO mice. Group mean + SD; n = 4–6. Columns with non-identical letters are statistically different at the significance level of $p < 0.05$. IA, interaction; NS, not significant.

change over the study period), at termination their body weight was still lower compared with that of males ($p < 0.001$; Fig. 3; Suppl. Table S1). No other differences among the groups existed as assessed by 3-way ANOVA. As a corollary, absolute (Suppl. Figs. S1 & S2) and relative organ weights (Fig. 3; Suppl. Figs. S1–S2) exhibited similar TCDD treatment-, sex- and genotype-related changes. There was a highly significant ($p < 0.001$) treatment x genotype interaction in relative liver weight with TCDD exposure increasing the weight in wild-type mice alone (TCDD-treated groups, wildtype *vs.* AHRKO: $p < 0.001$). Moreover, relative liver weight was conspicuously lower in control AHRKO *vs.* control wild-type mice ($p < 0.001$; Suppl. Table S2). In contrast, amongst untreated animals AHRKO mice had heavier kidneys than their wild-type counterparts ($p < 0.001$ for relative kidney weight; Suppl. Table S2). As expected, TCDD decreased thymus weight in wild-type

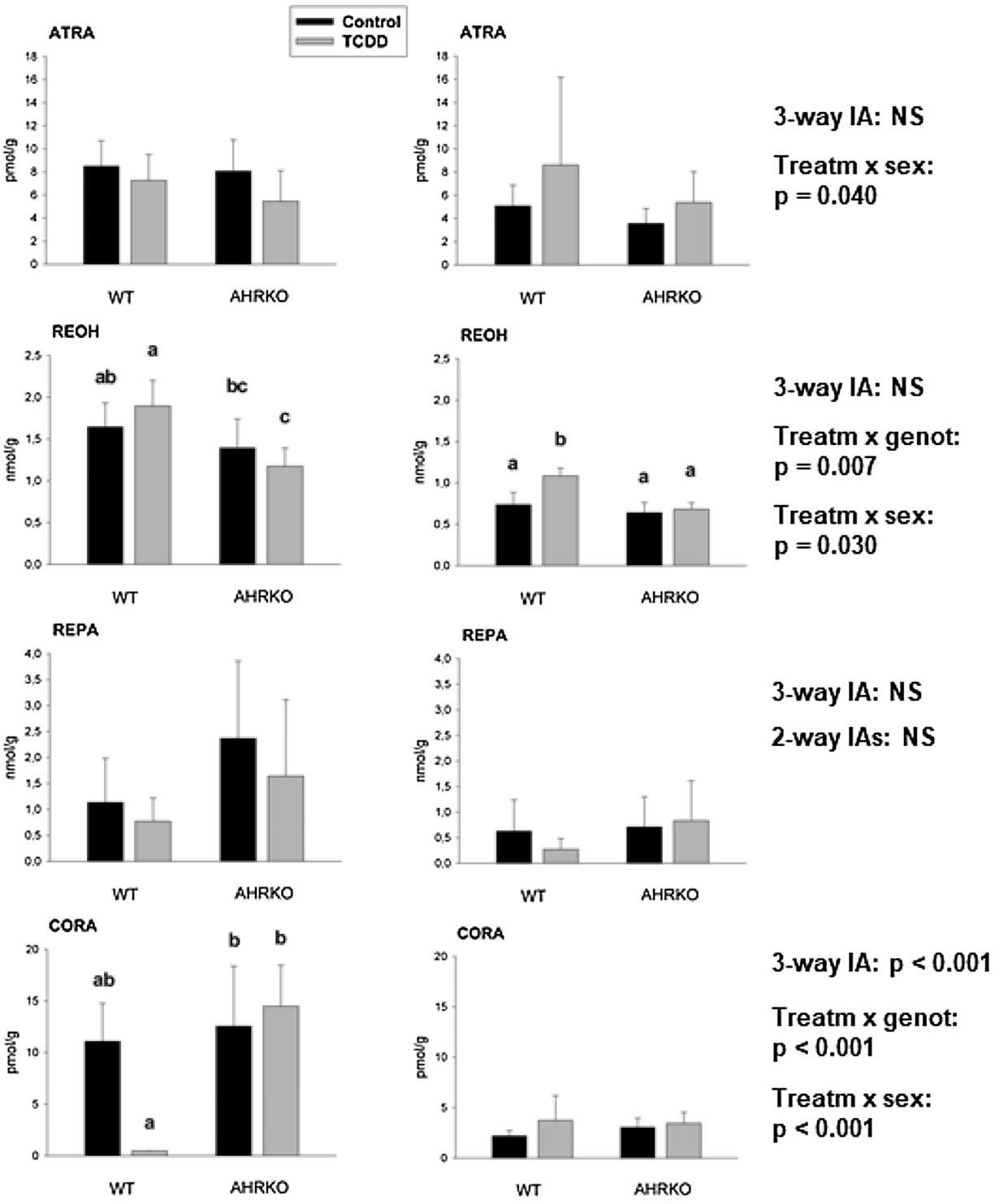

Fig. 6. Effects of TCDD on renal concentrations of ATRA, REOH, REPA and CORA in male (left panels) and female wild-type (WT) & AHRKO mice. Group mean + SD; n = 4–6 except for CORA in WT female controls, where n = 3. Columns with non-identical letters are statistically different at the significance level of $p < 0.05$. IA, interaction; NS, not significant.

mice alone (Fig. 3). Neither genotype nor TCDD dosing exhibited a statistically significant influence on absolute or relative testis or epididymis weights, although a marginally significant treatment x genotype interaction term existed for absolute testis weight (Suppl. Fig. S1).

3.2. Tissue retinoid levels

3.2.1. Liver

Retinoid concentrations in the liver are shown in Fig. 4. Significant alterations in response to TCDD treatment were observed in wild-type mice, while concentrations in AHRKO mice did not differ from those in wild-type controls. TCDD administration caused an increase in ATRA in wild-type females and a slight, statistically non-significant increase in

males. The concentrations of CORA were strongly reduced in both sexes of TCDD-treated wild-type mice, down to the levels of only 1.7 % and 5.7 % of the control value in females and males, respectively. Similarly, REPA concentrations were substantially lowered by TCDD in wild-type mice, although a statistically significant difference in comparison with the respective wild-type control was only reached in females. For ATRA, CORA and REPA, ANOVA confirmed a significant treatment x genotype interaction term. In pooled controls of both genotypes, females had higher hepatic concentrations of REOH ($p = 0.010$) and REPA ($p = 0.011$) but lower ATRA levels ($p = 0.013$) than males (Suppl. Table S1).

When the contents of these retinoids in the whole liver were calculated, the outcome closely resembled that of their concentrations (Suppl. Fig. S3).

3.2.2. Serum

Retinoid concentrations in the serum are depicted in Fig. 5. In wild-type mice, TCDD treatment resulted in a significant increase in female REOH levels and a downward tendency in male CORA concentrations. Males also showed a trend towards elevated REOH concentration. TCDD did not affect any of the measured retinoid forms in AHRKO mice. However, there were significant differences between the untreated AHRKO *vs*. wild-type mice in ATRA ($p = 0.007$) and REOH ($p < 0.001$) concentrations, the levels being higher in wild-type animals. When the genotypes were pooled, a sex difference was manifest in ATRA concentrations of controls with females harboring higher levels ($p = 0.005$; Suppl. Table S1). Moreover, a significant interaction between TCDD treatment and mouse genotype was established for REOH (Fig. 5).

3.2.3. Kidney

Retinoid concentrations in the kidney are presented in Fig. 6. Similarly to the liver and serum, significant alterations after TCDD exposure were only observed in wild-type mice. The most conspicuous impact of TCDD was a drastic decline in CORA concentration in male mice, resembling the effect in the liver. Unlike in the liver, however, female mice were unaffected. Thus, in the case of kidney CORA concentration, the outcome depended critically on treatment, genotype and sex, which was confirmed by a highly significant interaction term of these three factors ($p < 0.001$). This was also true of renal CORA content (Suppl. Fig. S4). In addition to CORA, TCDD had an effect on REOH, whose concentration was elevated in wild-type females and also exhibited an upward tendency in the males of this genotype. Consequently, a highly significant interaction between treatment and genotype was observed in the ANOVA (Fig. 6). There were further marginally significant interactions between treatment and sex for REOH and ATRA. Among control animals (across genotypes), male mice harboured higher concentrations of all the retinoids analysed compared with females ($p < 0.001$ for all except for REPA, where $p = 0.014$; Suppl. Table S1).

The patterns for total amounts of these retinoids in the kidneys were again highly similar to those of their concentrations (Suppl. Fig. S4).

3.3. Observed and previously published liver retinoid concentrations in AHRKO rodents

Study design and hepatic retinoid concentrations determined in this study were compared with previously published data in AHRKO rodent models (Table 1). The listed studies represent three different AHRKO mouse lines and one AHRKO rat model [1–4,81] current study). The study protocols vary also in terms of sex analysed, age at tissue sampling, number of individuals analysed per group, retinoid forms analysed, and dietary vitamin A content, which was detailed in two studies alone (Table 1). The three previous mouse studies presented their data in a figure-format with the need to estimate the numbers for comparison with this study. One of those studies did not present data in a standard concentration format and it was therefore not possible to make detailed comparisons between absolute concentration values, not even for hepatic REPA concentrations that were analysed and presented in all five studies. A concluding main observation from the literature survey is clear: only one of the four AHRKO lines showed a clear genotype effect with 3-fold higher liver concentrations of REOH, REPA and ATRA in mice lacking the AHR. In addition, hepatic REOH concentrations showed small variations among studies, groups and sex, as did ATRA concentrations in the reported studies.

Table 1
Observed and published data on liver retinoid concentrations in AHRKO rodents.

Publication						Hepatic retinoid concentration			
Species; strain Original KO line	Sex	Age at start (weeks)	Study duration (days)	Age at sampling (weeks)	Genotype (n)	REOH (nmol/g)	REPA (nmol/g)	ATRA (pmol/g)	CORA (pmol/g)
Andreola et al. [1][1]	M	20	3	20	WT (3–5)	3.9	1140	130	
Mouse; C57BL6/ CNrX129/Sv	M	20	3	20	*Ahr* +/- (3−5)	6.3*	2380*	200*	
	M	20	3	20	*Ahr* -/- (3)	10.8*	3810*	470*	
Andreola et al. [2,3] [1]	M	3	21	6	WT (2)		267		
Mouse; C57BL6/ CNrX129/Sv	M	3	21	6	*Ahr* -/- (2)		572*		
	M	3	63	12	WT (2)		2191		
	M	3	63	12	*Ahr* -/- (2)		4573*		
	M	3	105	18	WT (2)		2705		
	M	3	105	18	*Ahr* -/- (2)		4287*		
Nishimura et al. [4][2]	M + F	GD 12.5	28	3	WT (4–5)	~50	~160		
Mouse; C57BL/6 J	M + F	GD 12.5	28	3	*Ahr* +/- (4−5)	~55	~145		
	M + F	GD 12.5	28	3	*Ahr* -/- (4−5)	~60	~175		
Esteban et al. 2020[3]	F	8–12	70	18–22	WT (6)	17	721	16	113
Mouse; C57BL/6					*Ahr* -/- (6)	15	833	11	92
	M	8–12	70	18–22	WT (6)	9	582	19	121
					Ahr -/- (6)	12	645	22	144
Pohjanvirta et al. [81][3]	M	7–8	165	31–32	WT (5)	3.6	59	13	8.9
Rat; Sprague-Dawley SAGE/Horizon	M	7–8	165	31–32	*Ahr*-/- (7)	3.5	81	13	10.5

Empty spaces: no data reported.
* Statistically significant difference *vs*. wildtype.
[1] Retinoid values extracted from original bar charts and converted from grams to molar by use of the molecular weights for each analyte.
[2] Retinoid values presented as “units/g liver” (not defined).
[3] Vitamin A concentration in the diet reported.

4. Discussion

4.1. Retinoid data in the regulatory context

The number of scientific reports which have addressed the retinoid system in relation to the toxicology of dioxins, other contaminants, or production chemicals is growing, and it is now well known that disruption of vitamin A homeostasis, and thereby tissue concentrations of various forms of retinoids, is a sensitive endpoint in toxicology, and a potentially significant contributor to a broad spectrum of toxicity outcomes (reviewed in [66–69,82,83]). Among early reports from regulatory agencies, which also are increasing in numbers, was the World Health Organization (WHO) Environmental Health Criteria document on dioxins [84], which reported on similarities between symptoms of dioxin exposure and symptoms of inappropriate vitamin A status. Hepatic vitamin A reduction was later included as an effect-biomarker for retinoid disruption in the WHO Toxic Equivalency Factor (TEF) system developed for the assessment of mixtures of persistent organic pollutants whose mode of action is based on AHR activation [85–87]. Later the Swedish Environmental Protection Agency developed a report on endocrine disruption in reproduction, which in addition to the estrogen, androgen, thyroid hormone, and steroidogenesis (EATS) pathways, also covered the retinoid system [88]. Currently, there are several ongoing international regulatory initiatives focusing on the retinoid system as an emerging target in toxicology including endocrine disruption as reviewed by Grignard et al. [6]. A Nordic working paper on male and female reproduction organ development and functions was recently published in support of these activities, and identified gaps and opportunities for future research and regulatory measures to establish suitable tools and procedures for data interpretation and toxicological screening, which also capture the retinoid system [7]. One important starting point for some of these activities was the OECD Detailed Review Paper 178 [89], which identified a number of pathways beyond EATS, including AHR and retinoic acid signaling, to be in need of additional regulatory attention. To support these regulatory efforts, we applied mode-of-action and weight-of-evidence types of analytical approaches to further evaluate and mine the obtained results. This additional evaluation of the obtained data on tissue concentrations of selected endogenous retinoid analytes is highly motivated as the selected retinoid analytes reflect both the metabolic and transcriptional roles of the retinoid system. Therefore, beyond the comparative evaluation and interpretation of the obtained retinoid concentration results in relation to previously published data obtained under the influence of AHR inactivation (4.2) and over-activation by TCDD (4.3), respectively, we also evaluated the obtained results in relation to the published literature with the aim to provide regulatory relevant mode-of-action (4.4) and weight-of-evidence (4.5) insights.

4.2. Retinoid status of AHRKO mice

To our knowledge, this is the first study reporting on circulating, hepatic, and renal retinoid concentrations in both male and female AHRKO mice. In addition, this is the first report on retinoid concentration data in the AHRKO mouse line developed in the laboratory of Dr. Chris Bradfield and co-workers [5]. Clearly, the lack of genotype effect on hepatic retinoid concentrations both in adult male and female mice of this AHRKO line in our study is in contrast to the reported sizeably (3-fold) elevated concentrations of ATRA, REOH, and REPA in the livers of adult male mice of the AHRKO line developed in the laboratory of Dr. Frank Gonzalez and co-workers [1,2,16]; Table 1). Instead, the hepatic retinoid data of AHRKO mice in the current study is more in line with findings in a third mouse line developed in the laboratory of Dr. Yoshiaki Fujii-Kuriyama [4,17], and in a rat AHRKO line (Pohjanvirta et al., submitted). In the mouse model, homozygous male *Ahr-/-* mice exhibited a modest (9.4 %) elevation of hepatic REPA concentration compared with their wild-type counterparts, whereas heterozygotes displayed an identical reduction. In the rat model, AHRKO rats fed on a standard diet had a 36.9 % higher hepatic REPA concentration than their wild-type controls, but their hepatic ATRA, CORA, and REOH concentrations were comparable to those in wildtype animals (Pohjanvirta et al., submitted), which is in line with the corresponding data from the current AHRKO mouse study (Table 1). It has been proposed that differences in the genetic approach, when developing the AHRKO mouse lines, can contribute to differences observed in their phenotypes [19]; a possibility which might be relevant also for the retinoid end-points analysed in the current study.

Additional impact on reported retinoid results from the different AHRKO lines may come from differences in dietary vitamin A content and age of the study subject; however, such information is not always provided in the study reports (Table 1). As demonstrated in Table 1, it is in particular the hepatic REPA and CORA concentrations that are reflective of variations in the dietary vitamin A content, while hepatic REOH and ATRA concentrations remain within a narrower range even in cases of variable dietary vitamin A status. Currently, there are no published data on retinoid concentrations in extra-hepatic tissues of mammalian AHRKO lines to compare with. However, it is clear from previously reported rodent wildtype data that the retinoid concentrations in kidneys and circulation measured in this study are largely comparable to published data [90,91]. In general, the data variations observed among different studies can be accounted for by sex, age, rodent species, and dietary vitamin A content, as well as factors related to study design, such as TCDD dosing scheme and study duration. However, results obtained in the present study are clearly suggestive of sex differences in retinoid concentrations in all three tissues analysed. In particular, there was a distinct difference in kidney concentrations with female mice having consistently lower concentrations than male mice in all four analysed retinoid forms. Clearly, this observation deserves further investigation, not least in the context of the complexities involved in endocrine disruption and reproductive toxicology end-points.

In this study, none of the analysed hepatic or renal retinoid concentrations were affected by AHR ablation neither in female nor in male mice. At first glance, these findings may give the impression that AHR does not have a major role in the physiological control of retinoid concentrations in central and metabolically active organs, such as the liver and kidneys. However, on a more detailed level, such a conclusion can only be drawn following additional investigations, which include impact-studies of AHR on the dynamics behind the whole-body regulation of the retinoid system; meaning that also the impact of AHR on the dynamics involved in retinoic acid metabolism and signaling on the cellular level needs to be addressed. It could well be that whole organ retinoid concentrations as analysed in the current study differ from concentrations in individual cell compartments. This is because different cell types within organs have their own profiles when it comes to the retinoid system. Profile differences could include basal concentrations of specific retinoid forms and their binding proteins, as well as basal activities and inducibilities of retinoid-specific as well as general enzymes that are involved in retinyl ester storage and/or retinoic acid metabolic and signaling pathways. As a result, there are differences among cell-types in retinoid kinetics and dynamics. For example, many organs host local stores of vitamin A in the form of retinyl esters in vitamin A-storing stellate cells[7] and/or in lipid droplets of parenchymal cells

[7] Stellate cells are dendritic cells present in most organs and are well known for their key roles in collagen synthesis as well as in storage of vitamin A, other fat-soluble vitamins, triglycerides, cholesterol, and fatty acids [93,95]. They play important roles in organ development and regeneration, as well as in pathological conditions, such as steatosis and fibrosis [93,95,144,145]. Stellate cells are known under many different names, including Ito cells, pericytes, fat-storing cells, lipocytes, interstitial cells, vitamin A-storing cells and stern cells.

[92–95]. It is also well known that there is an extensive and continuous turnover, redistribution, and excretion of the stored body pool of vitamin A within and among organs to maintain proper overall and cellular homeostasis, and also to cope with times of normal as well as extensive variations in dietary vitamin A intake (reviewed by [96,97]).

Thus, for these several types of reasons, additional knowledge delineating the impact of AHR ablation or over-activation on cellular retinoid-system processes should also be extended to include kinetic retinoid tracer and gene data in models which address dynamic retinoid system processes on cell and whole-body levels. Nevertheless, and despite the limitations in the design of the current study, we consider the observed reductions in circulating REOH and ATRA concentrations both in male and female AHR deficient mice in this study to suggest that AHR does have a role in the control of maintaining the overall retinoid homeostasis. In normal conditions, both REOH and ATRA in circulation are strictly maintained within homeostatic concentrations. It is only under circumstances, such as marked dietary vitamin A deficency, pharmacological retinoic acid treatment, or some toxicological exposures that measurable changes in circulating retinoid concentrations will be observed [97]. Therefore, the reductions of 41.3 and 36.1 %, respectively, in circulating REOH and ATRA concentrations in the adult AHRKO mice in this study deserve further investigation.

4.3. Retinoid status of AHRKO and wild-type mice following over-activation by TCDD

AHRKO mice in this study did not display any retinoid system changes in response to over-activation by TCDD (Figs. 4–6), thus providing strong evidence to the full dependency on AHR mediation for the observed retinoid system changes in TCDD-exposed wild-type mice. This finding is in line with the response to TCDD in a considerably more limited study, where a different AHRKO mouse line was used to analyse combined apolar hepatic retinoid concentrations in pooled liver samples from offspring aged 3 weeks, which were exposed to TCDD during fetal development *via* the dams ([4]; Table 1). In the present study, 10-week exposure to TCDD resulted in decreased hepatic REPA and CORA concentrations in both sexes of wild-type mice. These findings are in agreement with results from TCDD exposure studies in different species and at different life-stages demonstrating that AHR over-activation by TCDD typically leads to an inappropriate elevation of the overall retinoid metabolism, which is characterized by decreased concentrations of apolar retinoids, *i.e.* mainly retinyl esters, in the liver, and associated changes in various retinoid forms in some, but not all extrahepatic tissues, as well as increased excretion of retinoid metabolites *via* urine and bile [4,67,91,98]. Detailed kinetic investigations in the rat have demonstrated that TCDD initiates a lasting mobilization of stored retinoids from the large and slowly turning over stellate cell pool to the plasma compartment, followed by elimination *via* urine and feces [99]. The kinetic modelling revealed that the TCDD-induced mobilization from the retinoid stores is most likely preceded by transcriptional events [99], such as the well known induction by TCDD of numerous liver enzymes (reviewed by [41,100]).

A particularly interesting and novel finding in the wild-type mouse part of this study was the striking drop in renal CORA concentration and content caused by TCDD in male mice exclusively. In wild-type males, CORA concentrations were reduced by TCDD also in serum and liver, whereas females displayed a reduction only in CORA liver concentrations. Also other studies have identified CORA as an especially responsive retinoid form, which is drastically reduced in the liver not only in response to TCDD [101] or mixtures of dioxin-like compounds [102,103], but also to AHR agonists of low toxicity [80] and to the non-dioxin-like molecule PCB180 [104]. There are only few studies on the CORA-metabolite to date both in terms of occurrence and functional data. The mechanism by which TCDD interferes with CORA tissue concentrations is not yet known. Likewise, the CORA metabolic pathway and its exact relationship to ATRA metabolism and signalling are only beginning to be explored; CORA is the first 9-*cis*-configured endogenous isomer of retinoic acid that has been detected in tissues of several species, including humans [53]. The synthesis and chemistry of CORA has been published [52], and based on a limited biological characterization using the chicken limb bud and cell transfection studies, the present view is that CORA's biological activity resembles that of ATRA, for example in terms of gene regulation associated with retinoic acid receptors [55]. Additional characterization is needed to clarify if CORA, in addition to RARα and RARβ, also may play a role in the activation of RXRs [55,105,171].

It is tempting to speculate that the nearly depleted renal CORA concentrations in male mice may be involved in the pathogenesis of acute TCDD lethality as there is a reported sex divergence of male mice being over 10-fold more susceptible to the acute lethality of TCDD [106]. On the other hand, along the same line of speculation it can be assumed that the female-specific responses to TCDD exposure observed in the current study, *i.e.* increases in circulating and renal REOH along with hepatic ATRA concentrations in wild-type mice, may represent a protective type of response towards acute TCDD lethality.

4.4. Mechanistic interpretation of obtained retinoid concentration data

Together, the reported retinoid concentration data, and especially the markedly decreased hepatic REPA and almost depleted renal CORA concentrations following TCDD exposure, point to a critical role of overactivated AHR in the control of enzymes, which are involved in retinoic acid metabolism and retinoid storage, release, and distribution processing (Fig. 1). Indeed, it is striking that several of the highly TCDD-responsive CYP enzymes, *i.e.* CYP1A1, CYP1A2, and CYP1B1, as well as several glutathione transferases (GTs), uridinediphosphoglucuronosyl transferases (UGTs), and aldehyde dehydrogenases (ALDHs) [107–113], at the same time are key enzymes involved in retinoic acid synthesis and degradation [114–119]. These TCDD-inducible enzymes, often referred to as the "AHR battery genes" [100,120], are important members of the Phase 1 and II metabolizing enzymes [121–123], which play important roles in the cell by controlling the synthesis, degradation, and kinetics of numerous endogenous, as well as dietary and other exogenous molecules. Some of the AHR-controlled, and/or TCDD-inducible genes, are also controlled by retinoic acid *via* a retinoic acid response element (RARE) in the gene promoter region [124,125]. In particular, it is striking that the highly TCDD-inducible AHR battery gene CYP1B1 under physiological conditions spatially complements the retinoic acid synthesising enzyme retinal dehydrogenase (RALDH), and in this way can mediate embryofetal tissue development at specific sites through retinoic acid independently of RALDH [126]. Moreover, retinoid receptor agonists have been shown to potentiate the induction of CYP1A1 mRNA by TCDD *in vitro* [127].

In addition to the Phase I and II enzymes, AHR over-activation by TCDD also impacts on the expression and/or activity of enzymes and binding proteins specifically dedicated to retinoic acid synthesis and signaling, as well as retinyl ester storage, release, and distribution processes. Included among the TCDD-induced retinoic acid synthesis and signaling gene activations are repression of retinol dehydrogenase 9 [112], which converts REOH to retinal in the rate-limiting step of retinoic acid synthesis [128], induction of ALDH/RALDH ([109,129] [this issue] [110,112,113]), which converts retinal to retinoic acid [59,126,130,131], and repression of cellular retinoic acid-binding protein type 2 (CRABP2) ([129] [this issue]), which facilitates the translocation of retinoic acid from the cytoplasm to the nucleus [59,126,130]. Included among the TCDD-modulated gene activities which impact on retinyl ester storage, release, and distribution processes, are repressions of two major hepatic retinyl ester hydrolases, *i.e.* esterase 2 and carboxylesterase 3 [109], repression of lecithin:retinol acetyltransferase (LRAT) [132], which is the predominant enzyme responsible for REOH esterification and thus vitamin A storage, and repression of the cellular retinol-binding protein type 1 (CRBP1) [112], which is indispensable for

efficient retinyl ester synthesis and storage [133]. Finally, it is striking that retinoic acid has been reported to down-regulate the expression of central components of the AHR signaling machinery, *i.e.* AHR itself, and its partner and repressor proteins, *i.e.* the aryl hydrocarbon receptor nuclear translocator (ARNT) and aryl hydrocarbon receptor repressor (AHRR), respectively, although a canonical RARE has not been reported in those genes [124,134]. Together, these data suggest that a substantial part of the variations in tissue retinoid concentrations observed in this study can be accounted for by the combined activation and/or repression of the general high capacity and highly inducible Phase I and II enzymes on the one hand, and the more specific retinoic acid-metabolizing and retinoid storage enzymes, and binding proteins on the other hand. More specifically, we propose that critical events following AHR over-activation, *e.g.* by TCDD, include inductions and/or repressions of multiple metabolizing enzymes and binding proteins. In turn, these modulations can affect intracellular retinoic acid concentrations and expression of genes regulated by it, with the potential to eventually interfere with vital cell processes. Although no gene expression measurements were carried out in the present study, the data cited above from the Tijet et al. [112] study emanate from the same line of AHRKO and WT mice. Nevertheless, for firm conclusions to be drawn on this proposed sequence of molecular events, more studies, including more genes and quantitative information, are needed.

4.5. Organ weight results in relation to the retinoid data

We found, as expected, decreased liver and increased kidney weights in the AHRKO mice, which confirm and support a role of AHR in the control of liver and kidney development both in male and female mice as has been reported earlier in this line [40]. Although there were no changes in hepatic or renal retinoid concentrations in this AHRKO line, it is tempting to speculate, based on the observed reductions in circulating REOH and ATRA concentrations, that dynamic modulations of the retinoid system might be present in these mice on the cellular or tissue levels. Such a speculation is motivated, as it is well known that appropriate retinoic acid signaling is crucial in liver and kidney organogenesis as well as in the regeneration of these tissues [135,136,172]. Support for the speculation that decreased circulating retinoid concentrations may reflect a dynamic change in retinoid processing being present on the cellular and tissue levels, can be inferred from results presented in two independent human studies. A prospective study showed associations between decreased concentrations of circulating retinoic acid and severity of non-alcoholic fatty liver disease [137], while in an older study progressively worsening stages of a more severe hepatic disease, classified as normal, persistent hepatitis, fatty liver, alcoholic hepatitis, and chirrosis, were associated with decreasing hepatic concentrations of REOH and retinyl esters [138]. More recently, it was shown that hepatic stellate cells of human origin are susceptible to AHR over-activation by TCDD exposure in cell cultures [73], suggesting that background-exposures to persistent high-affinity AHR modulators may be contributing to fatty liver disease in human populations as well. Together these results indicate that retinoid concentrations in circulation and in tissues both have the potential to serve as markers for fatty liver disease. However, many more experimental as well as observational details need to be clarified before useful tools for predictions, preventions, or treatments can be established.

We also found that the two well-established organ weight indices of sustained AHR over-activation by TCDD exposure, *i.e.* elevated relative liver weight and diminished relative thymus weight, were observed in the TCDD-exposed wild-type mice, but not in the AHRKO mice of this study. These findings are well in line with the established knowledge that both the increased liver weight and thymic atrophy require the presence of a functional AHR [139,140]. It is also well known that TCDD-induced liver enlargement is connected to marked induction of xenobiotic-metabolizing enzymes, foremost CYP1A1, CYP1A2 and CYP1B1, along with hepatic steatosis [41], while a key role of the retinoid system in the pathophysiology of several hepatic diseases is only beginning to be explored and understood in relation to the toxicology of different categories of chemicals, including dioxins (reviewed by [67,74,141,142,173]). In this study, we did not perform any histological analyses of the organs collected for weight and/or retinoid concentration analyses. However, the pronounced decreases in hepatic REPA and CORA concentrations observed in the AHR over-activated wild-type mice are well in line with the pathological phenomena underlying progressive liver toxicity from fatty liver/steatosis to steatohepatitis, fibrosis, chirrosis, and eventually hepatocellular carcinoma as described in situations of compromised retinoid status [2,3,14,70–72], exposure to TCDD and related compounds [41,107,143], and partly also in the murine AHR ablation phenotype [5,40].

On the cellular level, it is well known that stellate cells, in addition to their key role in vitamin A storage [58], and their significant involvement in liver development and in wound healing [144,145], play important roles in pathological conditions such as steatosis and fibrosis [14,93,95,144,146]. Becker et al. [147] proposed that TCDD-induced depletion of hepatic retinoid stores, largely localized to the stellate cell population of the liver [132,148,149], is causally linked to enhanced cell proliferation, biliary fibrosis and cholangiosarcoma, which is the observed adverse outcome progression to cholangiosarcoma in carcinogenicity studies with TCDD in rats [150]. Further support for this proposal comes from the observation that several of the xenobiotic-metabolizing enzymes that are immediately induced upon TCDD-binding to AHR, also play key roles in retinoic acid synthesis (*e.g.* CYP1A1, CYP1B1 and ALDHs) and degradation/elimination reactions (*e.g.* UGTs and GTs). Of special note is the finding that CYP1B1 in stellate cells is abundant and highly inducible by TCDD [50,151], while CYP1A1 is neither inducible by TCDD nor abundant in stellate cells [152]. Together, these data suggest that the molecular initiating event (MIE) of the proposed adverse outcome pathway (AOP) for liver tumor promotion [147] impacts on retinoic acid homeostasis both in the hepatocyte and stellate cell compartments. Furthermore, on the molecular level it has been demonstrated that a large number of genes related to fat metabolism, a major task of the liver, are transcriptionally regulated by retinoic acid [124,153]. Intriguingly, AHR signaling also regulates hepatic lipid metabolism in mice *via*, *e.g.*, CD36, a major fatty acid transporter [154,155], and global AHR deficiency protects mice from high-fat diet-induced obesity and fatty liver (reviewed in [156]).

In contrast to the TCDD-induced liver pathology, which seems to be largely AHR-mediated, it appears as if the TCDD-induced reduction in thymus weight as observed also in the current study is a result of several parallel phenomena in addition to AHR-mediation [41]. Recent data emphasize the role of retinoic acid as a transcriptional regulator in the homeostasis of thymic epithelial cells, which is essential for their function and for normal thymopoiesis [157]. These results are in line with previous data demonstrating that TCDD in the thymus specifically targets the epithelial cell population [158]; thereby, these data propose that an interactive AHR–retinoic acid-mediated phenomenon could be directly involved in TCDD-induced thymic atrophy.

Clearly, data presented in this study call for more research on AHR–retinoid interactions, not only in liver and immune system physiology and pathology but also for human and animal health in general. By choosing TCDD as the model compound in this study we know that basically all organ systems will be affected depending on dose and time, as TCDD is targeting most tissues and brings about distinct, specific and sustained AHR over-activation that can be detrimental to any health outcome both in animals and humans. Although more detailed studies on the cellular and molecular levels are needed, it is prudent to propose that the findings of this study are largely representative of other tissues and thereby support the view that disruption of the retinoid system is a significant component in the broad scope of AHR-mediated biology and toxicology. As both AHR and RARs/RXRs are universal transcriptional regulators present in virtually all cells and tissues across vertebrate

species [159,160], it is also a cautious speculation that intact hormonal cross-talk between AHR and retinoic acid metabolism and signalling is of general relevance also in other species, including humans, and over the life-course. In a broader context of receptor-mediated toxicology, we propose, based on data from this study in combination with a large body of literature from the broad fields of cell biology, nutrition, and toxicology research, that the diet-derived and *in situ*-synthesized small retinoic acid molecule, together with its metabolic and transcriptional machinery, at the same time contributes to the control of, and is dependent on AHR in biology and toxicology, including endocrine disruption, over the life-course.

4.6. Conclusions

In the present study, we have demonstrated that AHR is necessary for normal concentrations of REOH and ATRA in the circulation of adult mice. Hepatic and renal retinoid concentrations were not influenced by AHR deficiency, and thus the elevated hepatic retinoid concentrations reported in another AHRKO mouse line [1–3] were not confirmed in this study. Furthermore, we have identified AHR as a prerequisite for the typical TCDD-induced reductions in hepatic REPA and CORA concentrations in both sexes, and in renal CORA concentration in male mice exclusively. In female mice, a functional AHR was found to be required for the TCDD-induced elevation of hepatic ATRA concentration and of renal and circulating REOH concentrations. Our data on organ weights in wild-type *vs*. AHRKO mice support a role of AHR in the control of liver and kidney development in male and female mice as previously reported [40]. Finally, we observed for the first time in adult mice a distinct sex difference in concentrations of several retinoid forms and in several tissues, with the difference being most notable in the markedly lower kidney concentrations of all analysed retinoid forms in female mice.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

We thank Janne Korkalainen, Ulla Naukkarinen and Arja Moilanen for excellent technical assistance. This work was supported by the European Commission under the project BoneTox (QLK4-CT-2002-02528) and the authors are solely responsible for the contents of this paper, which does not necessarily represent the opinion of the European Community. Dr. Esteban received a grant (Reference A/07/09165) from Deutscher Akademischer Austauschdienst (Bonn, Germany).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2021.02.004.

References

[1] F. Andreola, P.M. Fernandez-Salguero, M.V. Chiantore, M.P. Petkovich, F. J. Gonzalez, L.M. De Luca, Aryl hydrocarbon receptor knockout mice (AHR-/-) exhibit liver retinoid accumulation and reduced retinoic acid metabolism, Cancer Res. 57 (14) (1997) 2835–2838.
[2] F. Andreola, D.F. Calvisi, G. Elizondo, S.B. Jakowlew, J. Mariano, F.J. Gonzalez, et al., Reversal of liver fibrosis in aryl hydrocarbon receptor null mice by dietary vitamin A depletion, Hepatology 39 (1) (2004) 157–166.
[3] F. Andreola, G.P. Hayhurst, G. Luo, S.S. Ferguson, F.J. Gonzalez, J.A. Goldstein, et al., Mouse liver CYP2C39 is a novel retinoic acid 4-hydroxylase. Its down-regulation offers a molecular basis for liver retinoid accumulation and fibrosis in aryl hydrocarbon receptor-null mice, J. Biol. Chem. 279 (5) (2004) 3434–3438.
[4] N. Nishimura, J. Yonemoto, Y. Miyabara, Y. Fujii-Kuriyama, C. Tohyama, Altered thyroxin and retinoid metabolic response to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin in aryl hydrocarbon receptor-null mice, Arch. Toxicol. 79 (5) (2005) 260–267.
[5] J.V. Schmidt, G.H. Su, J.K. Reddy, M.C. Simon, C.A. Bradfield, Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 6731–6736.
[6] E. Grignard, H. Håkansson, S. Munn, Regulatory needs and activities to address the retinoid system in the context of endocrine disruption: the European viewpoint, Reprod. Toxicol. 93 (2020) 250–258.
[7] TemaNord, Retinoids in mammalian reproduction, with an initial scoping effort to identify regulatory methods, TemaNord (2020) 507, https://doi.org/10.6027/temanord2020-507. ISBN 978-92-893-6530-7 (pdf). ISBN 978-92-893-6531-4 (online).
[8] M.E. Hahn, S.I. Karchner, R.R. Merson, Diversity as opportunity: insights from 600 million years of AHR evolution, Curr. Opin. Toxicol. 2 (2017) 58–71.
[9] A. Poland, E. Clover, A.S. Kende, M. DeCamp, C.M. Giandomenico, 3, 4, 3', 4'-Tetrachloro azoxybenzene and azobenzene: potent inducers of aryl hydrocarbon hydroxylase, Science 194 (4265) (1976) 627–630.
[10] A. Poland, J.C. Knutson, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity, Annu. Rev. Pharmacol. Toxicol. 22 (1) (1982) 517–554.
[11] P.A. Harper, D.S. Riddick, A.B. Okey, Regulating the regulator: factors that control levels and activity of the aryl hydrocarbon receptor, Biochem. Pharmacol. 72 (3) (2006) 267–279.
[12] E.C. Hoffman, H. Reyes, F.F. Chu, et al., Cloning of a factor required for activity of the Ah (dioxin) receptor, Science 252 (5008) (1991) 954–958.
[13] M.N. Avilla, K.M.C. Malecki, M.E. Hahn, R.H. Wilson, C.A. Bradfield, The Ah receptor: adaptive metabolism, ligand diversity, and the xenokine model, Chem. Res. Toxicol. 33 (4) (2020) 860–879.
[14] C. Duval, E. Blanc, X. Coumoul, Aryl hydrocarbon receptor and liver fibrosis, Curr. Opin. Toxicol. 8 (2018) 8–13.
[15] ÁC. Roman, J.M. Carvajal-Gonzalez, J.M. Merino, S. Mulero-Navarro, P. M. Fernández-Salguero, The aryl hydrocarbon receptor in the crossroad of signalling networks with therapeutic value, Pharmacol. Ther. 185 (2018) 50–63.
[16] P. Fernandez-Salguero, T. Pineau, D.M. Hilbert, T. McPhail, S. Lee, S. Kimura, et al., Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding ah receptor, Science 268 (5211) (1995) 722–726.
[17] J. Mimura, K. Yamashita, K. Nakamura, M. Morita, T.N. Takagi, K. Nakao, et al., Loss of teratogenic response to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice lacking the ah (dioxin) receptor, Genes Cells 2 (10) (1997) 645–654.
[18] J.V. Schmidt, C.A. Bradfield, Ah receptor signaling pathways, Annu. Rev. Cell Dev. Biol. 12 (1) (1996) 55–89.
[19] G.P. Lahvis, C.A. Bradfield, *Ahr* null alleles: distinctive or different? Biochem. Phramacol. 56 (7) (1998) 781–787.
[20] P.M. Fernandez-Salguero, J.M. Ward, J.P. Sundberg, F.J. Gonzalez, Lesions of aryl-hydrocarbon receptor-deficient mice, Vet. Pathol. 34 (6) (1997) 605–614.
[21] A.K. Lund, M.B. Goens, N.L. Kanagy, M.K. Walker, Cardiac hypertrophy in aryl hydrocarbon receptor null mice is correlated with elevated angiotensin II, endothelin-1, and mean arterial blood pressure, Toxicol. Appl. Pharmacol. 193 (2) (2003) 177–187.
[22] E.A. Thackaberry, D.M. Gabaldon, M.K. Walker, S.M. Smith, Aryl hydrocarbon receptor null mice develop cardiac hypertrophy and increased hypoxia-inducible factor-1alpha in the absence of cardiac hypoxia, Cardiovasc. Toxicol. 2 (4) (2002) 263–274.
[23] K.P. Singh, R.W. Garrett, F.L. Casado, T.A. Gasiewicz, Aryl hydrocarbon receptor-null allele mice have hematopoietic stem/progenitor cells with abnormal characteristics and functions, Stem Cells Res. Dev. Ther. 20 (5) (2010) 769–784.
[24] I. Bravo-Ferrer, M.I. Cuartero, V. Medina, et al., Lack of the aryl hydrocarbon receptor accelerates aging in mice, FASEB J. 33 (11) (2019) 12644–12654.
[25] M. Veldhoen, K. Hirota, A.M. Westendorf, J. Buer, L. Dumoutier, J. Renauld, et al., The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins, Nature 453 (7191) (2008) 106–109.
[26] B.D. Abbott, J.E. Schmid, J.A. Pitt, A.R. Buckalew, C.R. Wood, G.A. Held, et al., Adverse reproductive outcomes in the transgenic ah receptor-deficient mouse, Toxicol. Appl. Pharmacol. 155 (1) (1999) 62–70.
[27] T. Baba, J. Mimura, N. Nakamura, N. Harada, M. Yamamoto, K. Morohashi, et al., Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction, Mol. Cell. Biol. 25 (22) (2005) 10040–10051.
[28] R. Robles, Y. Morita, K.K. Mann, et al., The aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse, Endocrinology 141 (1) (2000) 450–453.
[29] J.C. Benedict, T.M. Lin, I.K. Loeffler, R.E. Peterson, J.A. Flaws, Physiological role of the aryl hydrocarbon receptor in mouse ovary development, Toxicol. Sci. 56 (2) (2000) 382–388.
[30] J.C. Benedict, K.P. Miller, T.M. Lin, et al., Aryl hydrocarbon receptor regulates growth, but not atresia, of mouse preantral and antral follicles, Biol. Reprod. 68 (5) (2003) 1511–1517.
[31] T. Baba, Y. Shima, A. Owaki, et al., Disruption of aryl hydrocarbon receptor (AhR) induces regression of the seminal vesicle in aged male mice, Sex. Dev. 2 (1) (2008) 1–11.
[32] T.M. Lin, K. Ko, R.W. Moore, D.L. Buchanan, P.S. Cooke, R.E. Peterson, Role of the aryl hydrocarbon receptor in the development of control and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-exposed male mice, J. Toxicol. Environ. Health Part A 64 (2001) 327–342.
[33] S. Ohsako, N. Fukuzawa, R. Ishimura, et al., Comparative contribution of the aryl hydrocarbon receptor gene to perinatal stage development and dioxin-induced toxicity between the urogenital complex and testis in the mouse, Biol. Reprod. 82 (3) (2010) 636–643.
[34] G.P. Lahvis, S.L. Lindell, R.S. Thomas, R.S. McCuskey, C. Murphy, E. Glover, et al., Portosystemic shunting and persistent fetal vascular structures in aryl

hydrocarbon receptor-deficient mice, Proc. Natl. Acad. Sci. 97 (19) (2000) 10442–10447.
[35] A. Morales-Hernández, A. Nacarino-Palma, N. Moreno-Marín, et al., Lung regeneration after toxic injury is improved in absence of dioxin receptor, Stem Cell Res. 25 (2017) 61–71.
[36] N. Moreno-Marín, E. Barrasa, A. Morales-Hernández, et al., Dioxin receptor adjusts liver regeneration after acute toxic injury and protects against liver carcinogenesis, Sci. Rep. 7 (1) (2017) 10420. Published 2017 Sep 5.
[37] A.K. Mayer, M. Mahajnah, M.G. Thomas, Y. Cohen, A. Habib, M. Schulze, G.D. E. Maconachie, B. AlMoallem, E. De Baere, B. Lorenz, E.I. Traboulsi, S. Kohl, A. Azem, P. Bauer, I. Gottlob, R. Sharkia, B. Wissinger, Homozygous stop mutation in AHR causes autosomal recessive foveal hypoplasia and infantile nystagmus, Brain 142 (June (6)) (2019) 1528–1534.
[38] A. Chevallier, A. Mialot, J.M. Petit, P. Fernandez-Salguero, R. Barouki, X. Coumoul, M. Beraneck, Oculomotor deficits in aryl hydrocarbon receptor null mouse, PLoS One 8 (1) (2013) e53520.
[39] L. Juricek, J. Carcaud, A. Pelhaitre, T.T. Riday, A. Chevallier, J. Lanzini, N. Auzeil, O. Laprévote, F. Dumont, S. Jacques, F. Letourneur, C. Massaad, C. Agulhon, R. Barouki, M. Beraneck, X. Coumoul, AhR-deficiency as a cause of demyelinating disease and inflammation, Sci. Rep. 7 (August (1)) (2017) 9794.
[40] J.A. Harrill, R.R. Hukkanen, M. Lawson, G. Martin, B. Gilger, V. Soldatow, E. L. Lecluyse, R.A. Budinsky, J.C. Rowlands, R.S. Thomas, Knockout of the aryl hydrocarbon receptor results in distinct hepatic and renal phenotypes in rats and mice, Toxicol. Appl. Pharmacol. 272 (2) (2013) 503–518.
[41] R. Pohjanvirta, J. Tuomisto, Short-term toxicity of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin in laboratory animals: effects, mechanisms, and animal models, Pharmacol. Rev. 46 (4) (1994) 483–549.
[42] L.S. Birnbaum, J. Tuomisto, Non-carcinogenic effects of TCDD in animals, Food Addit. Contam. 17 (4) (2000) 275–288.
[43] P.K. Mandal, Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology, J. Comp. Physiol. B 175 (4) (2005) 221–230.
[44] D.W. Nebert, T.P. Dalton, The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis, Nat. Rev. Cancer 6 (12) (2006) 947–960.
[45] USEPA, EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, Vol. 1, 2012. February (EPA/600/R-10/038F).
[46] EFSA, Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food, EFSA J. 16 (2018) 5333.
[47] P.M. Fernandez-Salguero, D.M. Hilbert, S. Rudikoff, J.M. Ward, F.J. Gonzalez, Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity, Toxicol. Appl. Pharmacol. 140 (1) (1996) 173–179.
[48] J.M. Peters, M.G. Narotsky, G. Elizondo, P.M. Fernandez-Salguero, F.J. Gonzalez, B.D. Abbott, Amelioration of TCDD-induced teratogenesis in aryl hydrocarbon receptor (AhR)-null mice, Toxicol. Sci. 47 (1) (1999) 86–92.
[49] H. Jacobs, C. Dennefeld, B. Féret, et al., Retinoic acid drives aryl hydrocarbon receptor expression and is instrumental to dioxin-induced toxicity during palate development, Environ. Health Perspect. 119 (11) (2011) 1590–1595.
[50] J.A. Walisser, E. Glover, K. Pande, A.L. Liss, C.A. Bradfield, Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types, Proc. Natl. Acad. Sci. U. S. A. 102 (49) (2005) 17858–17863.
[51] Y. Hayashida, T. Kawamura, R. Hori-e, I. Yamashita, Retionic acid and its receptors are required for expression of aryl hydrocarbon receptor mRNA and embryonic development of blood vessel and bone in the medaka fish, Oryzias latipes, Zoolog Sci. 21 (5) (2004) 541–551.
[52] M. Dominguez, S. Alvarez, R. Alvarez, A.R. de Lera, Stereocontrolled synthesis of *(S)*-9-cis-4-oxo-13,14-dihydroretinoic acid, Tetrahedron (2012) 1756–1761.
[53] C.K. Schmidt, J. Volland, G. Hamscher, H. Nau, Characterization of a new endogenous vitamin A metabolite, Biochim.et Biophys. Acta (BBA)-Mol. Cell Biol. Lipids 1583 (2) (2002) 237–251.
[54] C.K. Schmidt, P. Högberg, N. Fletcher, C.B. Nilsson, C. Trossvik, H. Håkansson, et al., 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters the endogenous metabolism of all-trans-retinoic acid in the rat, Arch. Toxicol. 77 (7) (2003) 371–383.
[55] J.P. Schuchardt, D. Wahlström, J. Rüegg, N. Giese, M. Stefan, H. Hopf, et al., The endogenous retinoid metabolite S-4-oxo-9-*cis*-13, 14-dihydro-retinoic acid activates retinoic acid receptor signalling both *in vitro* and *in vivo*, FEBS J. 276 (11) (2009) 3043–3059.
[56] E.M. Wiseman, S. Bar-El Dadon, R. Reifen, The vicious cycle of vitamin a deficiency: a review, Crit. Rev. Food Sci. Nutr. 57 (17) (2017) 3703–3714.
[57] S. Bar-El Dadon, R. Reifen, Vitamin A and the epigenome, Crit. Rev. Food Sci. Nutr. 57 (11) (2017) 2404–2411.
[58] R. Blomhoff, H.K. Blomhoff, Overview of retinoid metabolism and function, J. Neurobiol. 66 (7) (2006) 606–630.
[59] G. Duester, Retinoic acid synthesis and signaling during early organogenesis, Cell 134 (6) (2008) 921–931.
[60] M. Mark, N.B. Ghyselinck, P. Chambon, Function of retinoic acid receptors during embryonic development, Nucl. Recept. Signal. 7 (2009) e002.
[61] M. Rhinn, P. Dollé, Retinoic acid signalling during development, Development 139 (5) (2012) 843–858.
[62] A. André, R. Ruivo, M. Gesto, L.F. Castro, M.M. Santos, Retinoid metabolism in invertebrates: when evolution meets endocrine disruption, Gen. Comp. Endocrinol. 208 (2014) 134–145.
[63] R.M. Evans, D.J. Mangelsdorf, Nuclear receptors, RXR, and the big bang, Cell 157 (1) (2014) 255–266.
[64] M. Handberg-Thorsager, J. Gutierrez-Mazariegos, S.T. Arold, et al., The ancestral retinoic acid receptor was a low-affinity sensor triggering neuronal differentiation, Sci. Adv. 4 (2) (2018) eaao1261. Published 2018 Feb 21.
[65] R.D. Kimbrough, The toxicity of polychlorinated polycyclic compounds and related chemicals, CRC Crit. Rev. Toxicol. 2 (1974) 445–498.
[66] K.A. Murphy, L. Quadro, L.A. White, The intersection between the aryl hydrocarbon receptor (AHR)- and retinoic acid-signaling pathways, Vitam. Horm. 75 (2007) 33–67.
[67] C.B. Nilsson, H. Håkansson, The retinoid signaling system-A target in dioxin toxicity, CRC Crit. Rev. Toxicol. 32 (3) (2002) 211–232.
[68] J. Novák, M. Beníšek, K. Hilscherová, Disruption of retinoid transport, metabolism and signaling by environmental pollutants, Environ. Int. 34 (6) (2008) 898–913.
[69] M.H. Zile, Vitamin A homeostasis endangered by environmental pollutants, Proc. Soc. Exp. Biol. Med. 201 (1992) 141–153. Review. Erratum in: Proc Soc Exp Biol Med 1992 Dec;20(3):319.
[70] M. Okuno, S. Kojima, K. Akita, R. Matsushima-Nishiwaki, S. Adachi, T. Sano, et al., Retinoids in liver fibrosis and cancer, Front. Biosci. 7 (2002) d2014–d2018.
[71] A. Yanagitami, S. Yamada, S. Yasui, T. Shimomura, R. Murai, K. Murawaki, et al., Retinoic acid receptor alpha dominant negative form causes steatohepatitis and liver tumors in transgenic mice, Hepatomegaly 40 (2004) 366–375.
[72] Y. Shirakami, S.-A. Lee, R.D. Clugston, W.S. Blaner, Hepatic metabolism of retinoids and disease associations, Biochim. Biophys. Acta 1821 (2012) 124–136.
[73] W.A. Harvey, K. Jurgensen, X. Pu, C.L. Lamb, K.A. Cornell, R.J. Clark, C. Klocke, Mitchell KA Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) increases human hepatic stellate cell activation, Toxicology 344-346 (February) (2016) 26–33.
[74] K. Yoshizawa, A. Heatherly, D.E. Malarkey, N.J. Walker, A. Nyska, A critical comparison of murine pathology and epidemiological data of TCDD, PCB126, and PeCDF, Toxicol. Pathol. 35 (7) (2007) 865–879.
[75] FELASA, C. Rehbinder, P. Baneux, D. Forbes, et al., Recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea pig and rabbit experimental units. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management, November 1995, Lab Anim. 30 (3) (1996) 193–208.
[76] M. Herlin, M. Finnilä, P. Zioupos, A. Aula, J. Risteli, H.M. Miettinen, M. Korkalainen, T. Jämsä, J. Tuukkanen, H. Håkansson, M. Viluksela, New insights to the role of aryl hydrocarbon receptor in bone phenotype and in dioxin-induced modulation of bone microarchitecture and material properties, Toxicol. Appl. Pharmacol. 273 (1) (2013) 219–226.
[77] M. Gibaldi, D. Perrier, Pharmacokinetics, Marcel Dekker Inc., New York, 1975, 329 p.
[78] L.S. Birnbaum, Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in congenic strains of mice which differ at the *Ah* locus, Drug Metab. Dispos. 14 (1) (1986) 34–40.
[79] C. Nilsson, P. Högberg, C. Trossvik, V. Azaıs-Braesco, W. Blaner, G. Fex, et al., 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin increases serum and kidney retinoic acid levels and kidney retinol esterification in the rat, Toxicol. Appl. Pharmacol. 169 (2) (2000) 121–131.
[80] S. Mahiout, J. Lindén, J. Esteban, I. Sánchez-Pérez, S. Sankari, L. Pettersson, H. Håkansson, R. Pohjanvirta, Toxicological characterisation of two novel selective aryl hydrocarbon receptor modulators in Sprague-Dawley rats, Toxicol. Appl. Pharmacol. 1 (July (326)) (2017) 54–65.
[81] R. Pohjanvirta, I. Karppinen, S.G. Velázquez, J. Esteban, H. Håkansson, Effect of a high-fat diet on factors related to energy balance and inflammation in AH receptor-deficient rats, Toxicol. Lett. (2019), 314S1:S1–S309 (abstract P-Late-12).
[82] A.H. Piersma, E.V. Hessel, Y.C. Staal, Retinoic acid in developmental toxicology: teratogen, morphogen and biomarker, Reprod. Toxicol. 72 (2017) 53–61.
[83] I.O. Shmarakov, Retinoid-xenobiotic interactions: the ying and the yang, Hepatobil Surg Nutr. 4 (4) (2015) 243–267.
[84] WHO/IPCS, IPCS Environment Health Criteria 88: Polychlorinated Dibenzo-Para-Dioxins and Dibenzofurans, World Health Organization, Geneva, 1989.
[85] U.G. Ahlborg, A. Brouwer, M.A. Fingerhut, et al., Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept, Eur. J. Pharmacol. 228 (4) (1992) 179–199.
[86] M. Van den Berg, L. Birnbaum, A.T. Bosveld, et al., Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife, Environ. Health Perspect. 106 (12) (1998) 775–792.
[87] M. Van den Berg, L.S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, et al., The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds, Toxicol. Sci. 93 (2) (2006) 223–241.
[88] P.E. Olsson, B. Borg, B. Brunström, H. Håkansson, E. Klasson-Wehler, Endocrine disrupting substances. Impairment of reproduction and development. Report 4859 from the Swedish Environmental Protection Agency, 1998, pp. 1–150.
[89] OECD, Detailed Review Paper State of the Science on Novel In Vitroand In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors. Series on Testing & Assessment No. 178, 2012 (ENV/JM/MONO (2012)23).
[90] P.A. Bank, K.L. Salyers, M.H. Zile, Effect of tetrachlorodibenzo-*p*-dioxin (TCDD) on the glucuronidation of retinoic acid in the rat, Biochim. et Biophys. Acta (BBA)-Gen. Subj. 993 (1) (1989) 1–6.
[91] A. Brouwer, H. Håkansson, A. Kukler, K. Van den Berg, U. Ahlborg, Marked alterations in retinoid homeostasis of sprague-dawley rats induced by a single i.p.

dose of 10 micrograms/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, Toxicology 16 (October (58)) (1989) 267–283.
[92] N.E. Nagy, K.B. Holven, N. Roos, et al., Storage of vitamin A in extrahepatic stellate cells in normal rats, J. Lipid Res. 38 (4) (1997) 645–658.
[93] H. Senoo, Y. Mezaki, M. Fujiwara, The stellate cell system (vitamin A-storing cell system), Anat. Sci. Int. 92 (4) (2017) 387–455.
[94] E. Yamada, K. Hirosawa, The possible existence of a vitamin A-storing cell system, Cell Struct. Funct. 1 (2) (1976) 201–204.
[95] K. Wake, Hepatic stellate cells: three-dimensional structure, localization, heterogeneity and development, Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci. 82 (4) (2006) 155–164.
[96] M.H. Green, J.B. Green, Vitamin A intake and status influence retinol balance, utilization and dynamics in rats, J. Nutr. 124 (12) (1994) 2477–2485.
[97] M.H. Green, J.B. Green, The use of model-based compartmental analysis to study vitamin A metabolism in a non-steady state, Adv. Exp. Med. Biol. 537 (2003) 159–172.
[98] S.K. Kelley, C.B. Nilsson, M.H. Green, J.B. Green, H. Håkansson, Use of model-based compartmental analysis to study effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on vitamin A kinetics in rats, Toxicol. Sci. 44 (1998) 1–13.
[99] S.K. Kelley, C.B. Nilsson, M.H. Green, J.B. Green, H. Håkansson, Mobilization of vitamin A stores in rats after administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: a kinetic analysis, Toxicol. Sci. 55 (2) (2000) 478–484.
[100] Q. Ma, Overview of AHR functional domains and the classical AHR signaling pathway: induction of drug metabolizing enzymes, in: R. Pohjanvirta (Ed.), The AH Receptor in Biology and Toxicology, John Wiley & Sons, Hoboken, NJ, USA, 2012, pp. 35–46.
[101] P. Högberg, C.K. Schmidt, N. Fletcher, C.B. Nilsson, C. Trossvik, A. Gerlienke Schuur, A. Brouwer, H. Nau, N.B. Ghyselinck, P. Chambon, H. Håkansson, Retinoid status and responsiveness to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice lacking retinoid binding protein or retinoid receptor forms, Chem. Biol. Interact. 156 (September (1)) (2005) 25–39.
[102] L.E. Elabbas, D. Borg, J. Esteban, X. Barber, W.J. Bowers, J. Nakai, G. Hamscher, H. Nau, A. Åkesson, H. Håkansson, Gestational and lactational exposure to environmental contaminants detected in Canadian arctic human populations alters retinoid homeostasis in rat offspring, J. Toxicol. Environ. Health 77 (2014) 223–245.
[103] J. Esteban, L.E. Elabbas, D. Borg, M. Herlin, A. Åkesson, X. Barber, G. Hamscher, H. Nau, W.J. Bowers, J.S. Nakai, M. Viluksela, H. Håkansson, Gestational and lactational exposure to the polychlorinated biphenyl mixture Aroclor 1254 modulates retinoid homeostasis in rat offspring, Toxicol. Lett. 229 (2014) 41–51, https://doi.org/10.1016/j.toxlet.2014.04.021.
[104] M. Viluksela, P. Heikkinen, L.T. van der Ven, et al., Toxicological profile of ultrapure 2,2',3,4,4',5,5'-heptachlorbiphenyl (PCB 180) in adult rats, PLoS One 9 (8) (2014) e104639. Published 2014 Aug 19.
[105] R. Rühl, A. Krzyżosiak, A. Niewiadomska-Cimicka, et al., 9-cis-13,14-Dihydroretinoic acid is an endogenous retinoid acting as RXR ligand in mice, PLoS Genet. 11 (6) (2015) e1005213.
[106] R. Pohjanvirta, H. Miettinen, S. Sankari, N. Hegde, J. Lindén, Unexpected gender difference in sensitivity to the acute toxicity of dioxin in mice, Toxicol. Appl. Pharmacol. 262 (July (2)) (2012) 167–176.
[107] D.R. Boverhof, L.D. Burgoon, C. Tashiro, et al., Temporal and dose-dependent hepatic gene expression patterns in mice provide new insights into TCDD-mediated hepatotoxicity, Toxicol. Sci. 85 (2) (2005) 1048–1063.
[108] D.R. Boverhof, L.D. Burgoon, C. Tashiro, et al., Comparative toxicogenomic analysis of the hepatotoxic effects of TCDD in Sprague Dawley rats and C57BL/6 mice, Toxicol. Sci. 94 (2) (2006) 398–416.
[109] N. Fletcher, D. Wahlström, R. Lundberg, C.B. Nilsson, K.C. Nilsson, K. Stockling, et al., 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters the mRNA expression of critical genes associated with cholesterol metabolism, bile acid biosynthesis, and bile transport in rat liver: a microarray study, Toxicol. Appl. Pharmacol. 207 (1) (2005) 1–24.
[110] A.K. Kopec, D.R. Boverhof, L.D. Burgoon, et al., Comparative toxicogenomic examination of the hepatic effects of PCB126 and TCDD in immature, ovariectomized C57BL/6 mice, Toxicol. Sci. 102 (1) (2008) 61–75.
[111] A.K. Kopec, M.L. D'Souza, B.D. Mets, et al., Non-additive hepatic gene expression elicited by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) co-treatment in C57BL/6 mice, Toxicol. Appl. Pharmacol. 256 (2) (2011) 154–167.
[112] N. Tijet, P.C. Boutros, I.D. Moffat, A.B. Okey, J. Tuomisto, R. Pohjanvirta, Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries, Mol. Pharmacol. 69 (1) (2006) 140–153.
[113] H. Toyoshiba, T. Yamanaka, H. Sone, F.M. Parham, N.J. Walker, J. Martinez, et al., Gene interaction network suggests dioxin induces significant linkage between aryl hydrocarbon receptor and retinoic acid receptor beta, Environ. Health Perspect. 112 (2004) 1217–1224.
[114] E.S. Roberts, A.D. Vaz, M.J. Coon, Role of isozymes of rabbit microsomal cytochrome P-450 in the metabolism of retinoic acid, retinol, and retinal, Mol. Pharmacol. 41 (2) (1992) 427–433.
[115] G.M. Raner, A.D. Vaz, M.J. Coon, Metabolism of all-trans, 9-cis, and 13-cis isomers of retinal by purified isozymes of microsomal cytochrome P450 and mechanism-based inhibition of retinoid oxidation by citral, Mol. Pharmacol. 49 (March (3)) (1996) 515–522.
[116] P.A. Spear, H. Garcin, J.F. Narbonne, Increased retinoic acid metabolism following 3,3',4,4',5,5'-hexabromobiphenyl injection, Can. J. Physiol. Pharmacol. 66 (September (9)) (1988) 1181–1186.
[117] S. Tomita, E. Okuyama, T. Ohnishi, Y. Ichikawa, Characteristic properties of a retinoic acid synthetic cytochrome P-450 purified from liver microsomes of 3-methylcholanthrene-induced rats, Biochim. Biophys. Acta 1290 (August (3)) (1996) 273–281.
[118] H. Chen, W.N. Howald, M.R. Juchau, Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human P-450 cytochromes, Drug Metab. Dispos. 28 (3) (2000) 315–322.
[119] Q.Y. Zhang, D. Dunbar, L. Kaminsky, Human cytochrome P-450 metabolism of retinals to retinoic acids, Drug Metab. Dispos. 28 (March (3)) (2000) 292–297.
[120] C. Köhle, K.W. Bock, Coordinate regulation of Phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2, Biochem. Pharmacol. 73 (12) (2007) 1853–1862.
[121] K. Abraham, R. Krowke, D. Neubert, Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin - 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin *O*-deethylase in rats following a single injection, Arch. Toxicol. 62 (1988) 359–368.
[122] W. Hu, C. Sorrentino, M.S. Denison, K. Kolaja, M.R. Fielden, Induction of cyp1a1 is a nonspecific biomarker of aryl hydrocarbon receptor activation: results of large scale screening of pharmaceuticals and toxicants in vivo and in vitro, Mol. Pharmacol. 71 (6) (2007) 1475–1486.
[123] D.W. Nebert, T.P. Dalton, A.B. Okey, F.J. Gonzalez, Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer, J. Biol. Chem. 279 (23) (2004) 23847–23850.
[124] J.E. Balmer, R. Blomhoff, Gene expression regulation by retinoic acid, J. Lipid Res. 43 (2002) 1773–1808.
[125] F. Vecchini, M.C. Lenoir-Viale, C. Cathelineau, J. Magdalou, B.A. Bernard, B. Shroot, Presence of a retinoid responsive element in the promoter region of the human cytochrome P4501A1 gene, Biochem. Biophys. Res. Commun. 201 (3) (1994) 1205–1212.
[126] D. Chambers, L. Wilson, M. Maden, A. Lumsden, RALDH-independent generation of retinoic acid during vertebrate embryogenesis by CYP1B1, Development 134 (7) (2007) 1369–1383.
[127] S. Hessel-Pras, A. Ehlers, A. Braeuning, A. Lampen, The aryl hydrocarbon receptor and retinoid receptors cross-talk at the CYP1A1 promoter *in vitro*, EXCLI J. 15 (March (17)) (2018) 246–256.
[128] X. Li, Y. Dai, P.Y. Chuang, J.C. He, Induction of retinol dehydrogenase 9 expression in podocytes attenuates kidney injury, J. Am. Soc. Nephrol. 25 (9) (2014) 1933–1941.
[129] M. Herlin, J. Esteban, M. Korkalainen, X. Barber, M. Finnilä, B. Joseph, M. Viluksela, H. Håkansson, Retinoic Acid Metabolism and Signaling in the Bone Toxicity Phenotype Induced by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, 2020. Manuscript to be submitted to the same Special Issue.
[130] M. Maden, Retinoid signalling in the development of the central nervous system, Nat. Rev. Neurosci. 3 (11) (2002) 843–853.
[131] S. Kumar, L.L. Sandell, P.A. Trainor, F. Koentgen, G. Duester, Alcohol and aldehyde dehydrogenases: retinoid metabolic effects in mouse knockout models, Biochim. Biophys. Acta 1821 (1) (2012) 198–205.
[132] C.B. Nilsson, A. Hanberg, C. Trossvik, Håkansson H. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin affects retinol esterification in rat hepatic stellate cells and kidney, Environ. Toxicol. Pharmacol. 2 (August (1)) (1996) 17–23.
[133] N.B. Ghyselinck, C. Båvik, V. Sapin, M. Mark, D. Bonnier, C. Hindelang, A. Dierich, C.B. Nilsson, H. Håkansson, P. Sauvant, V. Azaïs-Braesco, M. Frasson, S. Picaud, P. Chambon, Cellular retinol-binding protein I is essential for vitamin A homeostasis, EMBO J. 18 (September (18)) (1999) 4903–4914.
[134] R. Wanner, A. Panteleyev, B.M. Henz, T. Rosenbach, Retinoic acid affects the expression rate of the differentiation-related genes aryl hydrocarbon receptor, ARNT and keratin 4 in proliferative keratinocytes only, Biochim. Biophys. Acta 1317 (2) (1996) 105–111.
[135] T. Gilbert, Vitamin A and kidney development, Nephrol. Dial. Transplant. 17 (Suppl. 9) (2002) 78–80.
[136] S.K. Mallipattu, J.C. He, The beneficial role of retinoids in glomerular disease, Front. Med. (Lausanne) 2 (16) (2015). Published 2015 Mar 23.
[137] Y. Liu, H. Chen, J. Wang, W. Zhou, R. Sun, M. Xia, Association of serum retinoic acid with hepatic steatosis and liver injury in nonalcoholic fatty liver disease, Am. J. Clin. Nutr. 102 (1) (2015) 130–137.
[138] M.A. Leo, C.S. Lieber, Hepatic vitamin A depletion in alcoholic liver injury, N. Engl. J. Med. 307 (10) (1982) 597–601.
[139] M.K. Bunger, S.M. Moran, E. Glover, T.L. Thomae, G.P. Lahvis, B.C. Lin, C. A. Bradfield, Resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor, J. Biol. Chem. 278 (May (20)) (2003) 17767–17774.
[140] M.K. Bunger, E. Glover, S.M. Moran, J.A. Walisser, G.P. Lahvis, E.L. Hsu, C. A. Bradfield, Abnormal liver development and resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity in mice carrying a mutation in the DNA-binding domain of the aryl hydrocarbon receptor, Toxicol. Sci. 106 (November (1):8) (2008) 3–92.
[141] W.S. Blaner, Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders, Pharmacol. Ther. 197 (2019) 153–178.
[142] G. Shiota, H. Tsuchiya, Y. Hoshikawa, The liver as a target organ of retinoids, Hepat Res. 36 (4) (2006) 248–254.
[143] R. Nault, K.A. Fader, D.A. Ammendolia, P. Dornbos, D. Potter, B. Sharratt, et al., Dose-dependent metabolic reprogramming and differential gene expression in TCDD-elicited hepatic fibrosis, Toxicol. Sci. 154 (2) (2016) 253–266.
[144] R. Carmona, S. Barrena, R. Muñoz-Chápuli, Retinoids in stellate cells: development, repair, and regeneration, J. Dev. Biol. 7 (May (2)) (2019) pii: E10.

[145] M. Coll, L. Perea, R. Boon, et al., Generation of hepatic stellate cells from human pluripotent stem cells enables in vitro modeling of liver fibrosis, Cell Stem Cell 23 (1) (2018) 101–113, e7.
[146] J. Li, Y.R. Zhao, Z. Tian, Roles of hepatic stellate cells in acute liver failure: from the perspective of inflammation and fibrosis, World J. Hepatol. 11 (May (5)) (2019) 412–420.
[147] R.A. Becker, G. Patlewicz, T.W. Simon, J.C. Rowlands, R.A. Budinsky, The adverse outcome pathway for rodent liver tmor promotion by sustained activation of the aryl hydrocarbon receptor, Regul. Toxicol. Pharmacol. 73 (2015) 172–190.
[148] H. Hanberg, L. Kling, H. Håkansson, Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic stellate cell population in the rat, Chemosphere 32 (1996) 1225–1233.
[149] H. Håkansson, A. Hanberg, U.G. Ahlborg, The distribution of ^{14}C-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) between parenchymal and non-parenchymal rat hepatic cells and its effect on the vitamin A content of these cells, Chemosphere 18 (1989) 307–312.
[150] NTP, NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (gavage studies), Toxicol. Program Tech. Rep. Ser. (April (521)) (2006) 4–232.
[151] F. Piscaglia, T. Knittel, D. Kobold, S. Barnikol-Watanabe, Roccop Di, G. Ramadori, Cellular localization of hepatic cytochrome 1B1 expression and its regulation by aromatic hydrocarbons and inflammatory cytokines, Biochem. Pharmacol. 58 (1999) 157–165.
[152] D.L. Alexander, S.E. Eltom, C.R. Jefcoate, Ah receptor regulation of CYP1B1 expression in primary mouse embryo-derived cells, Cancer Res. 57 (1997) 4498–4506.
[153] M.M. McGrane, Vitamin A regulation of gene expression: molecular mechanism of a prototype gene, J. Nutr. Biochem. 18 (2007) 497–508.
[154] Y. Kawano, S. Nishiumi, S. Tanaka, K. Nobutani, A. Miki, Y. Yano, Y. Seo, H. Kutsumi, H. Ashida, T. Azuma, M. Yoshida, Activation of the aryl hydrocarbon receptor induces hepatic steatosis via the upregulation of fatty acid transport, Arch. Biochem. Biophys. 504 (December (2)) (2010) 221–227.
[155] J.H. Lee, T. Wada, M. Febbraio, J. He, T. Matsubara, M.J. Lee, F.J. Gonzalez, W. Xie, A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis, Gastroenterology 139 (2) (2010) 653–663.
[156] R. Pohjanvirta, AHR in energy balance regulation, Curr. Opin. Toxicol. 2 (2017) 8–14.
[157] K. Wendland, K. Niss, K. Kotarsky, et al., Retinoic acid signaling in thymic epithelial cells regulates thymopoiesis, J. Immunol. 201 (2) (2018) 524–532.
[158] W.F. Greenlee, K.M. Dold, R.D. Irons, R. Osborne, Evidence for direct action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thymic epithelium, Toxicol. Appl. Pharmacol. 79 (1) (1985) 112–120.
[159] F. Campo-Paysaa, F. Marlétaz, V. Laudet, M. Schubert, Retinoic acid signaling in development: tissue-specific functions and evolutionary origins, Genesis 46 (11) (2008) 640–656.
[160] M.E. Hahn, Aryl hydrocarbon receptors: diversity and evolution, Chem. Biol. Interact. 141 (1–2) (2002) 131–160.
[161] ÁR. de Lera, W. Krezel, Rühl R. An endogenous mammalian retinoid X. Receptor ligand, at last!, ChemMedChem 11 (10) (2016) 1027–1037.
[165] UNEP - The United Nations Environment Programme, Text of the Stockholm Convention on Persistent Organic Pollutants for Adoption by the Conference of Plenipotentiaries, 2001 (amended in 2009).
[166] S.A. Ross, P.J. McCaffery, U.C. Drager, L.M. De Luca, Retinoids in embryonal development, Physiol. Rev. 80 (3) (2000) 1021–1054.
[167] IUPAC-IUB, Joint commission on biochemical nomenclature (JCBN). Nomenclature of retinoids, Arch. Biochem. Biophys. 224 (1983) 728–731.
[168] F. Villarroya, M. Giralt, R. Iglesias, Retinoids and adipose tissues: metabolism, cell differentiation and gene expression, Int. J. Obes. Relat. Metab. Disord. 23 (1) (1999) 1–6.
[169] Håkansson H (2020) Vitamin A and the retinoid system – from nutrition to endocrine disruption. In: Challenges in Endocrine Disruptor Toxicology and Risk Assessment. Eds Mantovani A, Fucic A. pp. 268-280 Link to book: https://pubs.rsc.org/en/content/ebook/978-1-78801-741-1.
[170] N. Tijet, P.C. Boutros, I.D. Moffat, A.B. Okey, J. Tuomisto, R. Pohjanvirta, Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries, Mol. Pharmacol. 69 (1) (2006) 140–153.
[171] Á.R. de Lera, W. Krezel, R. Rühl, An Endogenous Mammalian RetinoidÁX Receptor Ligand, At Last!, Chem. Med. Chem. 11 (10) (2016) 1027–1037.
[172] R. Carmona, S. Barrena, R. Muñoz-Chápuli, Retinoids in Stellate Cells: Development, Repair, and Regeneration, J. Dev. Biol. 7 (2) (2019) 10.
[173] Y. Shirakami, S.-A. Lee, R.D. Clugston, W.S. Blaner, Hepatic metabolism of retinoids and disease associations, Biochim. Biophys. Acta. 1821 (2012) 124–136.
[174] M. Theodosiou, V. Laudet, M. Schubert, From carrot to clinic: an overview of the retinoic acid signaling pathway, Cell. Mol. Life Sci. 67 (9) (2010) 1423–1445.
[175] J.A. Walisser, E. Glover, K. Pande, A.L. Liss, C.A. Bradfield, Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types, Proc. Natl. Acad. Sci. U S A 102 (49) (2005) 17858–17863.