# Biological and Toxicological Responses to Dioxins Exposures

Livia Malorni[1]*, Rosaria Cozzolino[2]*, Floriana Boscaino[3]*, Luca Malorni[4], Giuseppe Palmieri[5], Antonio Malorni[6]*

[1,2,3,6]Proteomic and Biomolecular Mass Spectrometry Center, Institute of Food Science and Technology, National Research Council (CNR), Via Roma 64, I-83100 Avellino, Italy; Ph. +39 0825 299111; Fax: +39 0825 781585;
[4]Oncology Unit "Sandro Pitigliani", Nuovo Ospedale di Prato Santo Stefano, Via Suor Niccolina - 59100 Prato Italy; Ph. 347 6876562;
[4]Breast Center at Baylor College of Medicine One Baylor Plaza- 77030 Houston - TX
[5]Unit of Cancer Genetics, Institute of Biomolecular Chemistry, National Research Council (CNR), Traversa La Crucca 3, Baldinca Li Punti, I-07100 Sassari; Ph. +39 339 2800855;

***Abstract—*** *After the II Word War, the chemical based industrial revolution generated a wide and global contamination due to the release in the environment of thousand of compounds without an adequate knowledge of their environmental biotransformation and their toxic effect on the living matter. Recently, it has been found that several of these compounds and/or their relative by-products are persistent environmental contaminants associated with undesirable long-term effects. At present many questions have to be clarified with particular reference to lipophilic polyhalogenated compounds, such as polychloro-dibenzo-dioxins (PCDD), polychloro-dibenzo-furans (PCDF) and polychloro-biphenyls (PCB). These compounds accumulate up the food chain and humans can reach relative high concentration in their body with a consequent risk for health. In this paper we discuss the some basic features of both biological and toxicological aspects related to the dioxins exposure.*

***Keywords— Dioxins, AhR, Estrogens.***

## I. INTRODUCTION

The identification of the Aryl hydrocarbon Receptor (AhR) [Poland et al., 1982] and the finding that it mediates the toxic effects of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), has stimulated a large number of studies and posed several questions that, in some cases, have not yet led to a definitive conclusion. For this reason, the classification of the responses to exposure to low doses of dioxins as *biological* or *toxicological* is still arbitrary. For example, the induction of gene expression, controlled by AhR, of specific enzymes capable, in turn, to interact with other transcription factors and membrane receptors, is considered by some as an adaptive response, which is necessary to minimize the toxicity of ubiquitous environmental contaminants, and by others as a toxicological damage that can lead to a number of pathological conditions, including some extremely serious. Recently, a consistent amount of evidence indicated that some functions of the immune system might be AHR-dependent [Stevens et al., 2009]. Indeed, activation of the AHR signalling pathway seems to promote both differentiation of the regulatory T-cells (Treg) and proliferation of the T-helper 17 (Th17) cells, thus inducing immunosuppression and inflammation (through increased secretion of inflammatory cytokines), respectively [Kimura et al., 2008; Quintana et al., 2008; Stevens et al., 2009].

AhR is a transcriptional regulator that is highly conserved during the evolution. It is expressed in vertebrates and in invertebrates and it is linked to different functions. For example, in the nematode worm *Caenorhabditis elegans* AhR is involved in nervous system development, while in *Drosophila* it plays a role in the growth of the antennae, legs and in the colour vision [Schmidt et al., 1996]. In mammals, the physiological functions of AhR came mainly from studies in knockout mice [Schmidt et al., 1996; Fernandez-Salguero et al., 1997; Mimura et al., 1997]. These genetically modified mice, in which the expression of the AhR gene is deleted, show a reduced fertility, a lower liver volume presumably due to a vascular defect [Schmidt et al., 1996], and portal fibrosis.

The high degree of AhR conservation along the evolutionary scale and the observations conducted on mice with mutant phenotypes suggest that this receptor has a role beyond that of mediating the toxicity of xenobiotics. Recent studies have suggested, in fact, that the toxicity mediated by dioxins may reflect the dysregulation of the endogenous function of AhR. These substances, very persistent and stable, could lead to prolonged and inappropriate stimuli, causing a sort of continuous and improper activation of the AHR-dependent signal transduction [Stockinger, 2009; Stevens et al., 2009].

TCDD is the most studied congener of dioxins, which include both the family of polychlorinated dibenzodioxins (PCDDs) and that of polychlorinated dibenzofurans (PCDFs), and other dioxin-like environmental contaminants, such as polychlorinated biphenyls-(PCB). Because of their wide dispersal and long persistence in the environment, these substances

have generated great interest not only in scientific research but also in the general population, as a consequence of environmental contamination episodes due to industrial accidents (Seveso, 1976) or food contamination (Belgian chickens, June 1999).

Experimental studies on animals and on human cell lines, in addition to epidemiological studies conducted on populations accidentally exposed to dioxins, have revealed that the toxic responses are species-specific and are related to AhR polymorphism. In the case of human exposure to high-dose, the most commonly observed physio-pathological response is the chloracne [JECFA, 2001; Sorg et al., 2009]. This has been also confirmed by the monitoring of the Seveso population, which was exposed to high doses of dioxins in 1976 for the explosion of a reactor of the chemical industry ICMESA [Bertazzi et al., 1996].

The exposure to high doses shows an epidemiological association with other non-cancer health effects, such as an increased activity of hepatic enzymes, cardiovascular diseases, diabetes, thyroid dysfunction, effects on growth. However, most of these effects, such as chloracne, appear only at doses higher of several orders of magnitude than those to which humans are normally exposed (background of food contamination) [JECFA, 2001]. Finally, in studies focusing on high dose exposures, the pattern of exposure does not reflect that of studies accounting for low dose exposures and long term diet.

## II. PROPERTIES, DISTRIBUTION, EXPOSURE AND PCDD, PCDF AND PCB LEVELS

Speaking about "dioxins" from a toxicological point of view, we refer to 419 distinct compounds, which differ by the number of chlorine atoms in the molecule and which belong to three distinct families: polychlorinated dibenzo-p-dioxins (PCDDs, 75 different congeners), polychlorinated dibenzo-furans (PCDF, 135 different congeners) and polychlorinated biphenyls (PCBs, 209 different congeners). These latter are industrial products and have been mainly used for decades as dielectric in electrical transformers. Although their use has been abandoned, problems related to their previous environmental release in large quantities are still present, becoming the most common contaminants on Earth. In fact, PCBs are widely disperse in the environment, so much that they are present in the air sampled over non-contaminated areas at levels of 0.002 ng/$m^3$ [Polychlorinated biphenyls and terphenyls, 2nd ed. Geneva, World Health Organization, 1993 (Environmental Health Criteria, No. 140)].

Conversely, compounds belonging to the first two families have not been intentionally created by man and are dispersed into the environment by natural causes or involuntarily, as a result of human activities. In fact, one of the major sources of dioxin emissions is the burning of forests, which is a natural event occurred before the appearance of humans on Earth. Recently, dioxins have been found in kaolin, demonstrating a geological natural origin of such compounds. The average amount of octachloro-dibenzo-p-dioxin measured in the American kaolin is about 400 ng/g dry weight against an average amount of 100 ng/g measured in the fly ashes of incinerators [Horii et al., 2008]. This means that the animal life has arose and evolved since its beginnings in an environment in which dioxins were present. In fact, a great deal of dioxins is formed during natural abiogenic processes, such as volcanoes, forest fires, and other geothermal processes. But dioxins are also produced by living organisms. The oceans are the single largest source of biogenic organohalogens, which are biosynthesized by myriad seaweeds, sponges, corals, tunicates, bacteria, and other marine life. Spongiadioxins A (Fig. 1), B and C, produced by the Australian sponge *Dysidea dendyi* as repellents, and other dioxins with strong antifungal activity, produced by other types of sponges and by different fungi, are well known. Currently, pharmacological researches are under way, aimed to using these compounds for their broad-spectrum antifungal actions (mainly, Candida infections) and for their possible antimalarial activities. Finally, terrestrial plants, fungi, lichen, bacteria, insects, some higher animals, and even humans also account for a diverse collection of organohalogens. Since human myeloperoxidase (MPO), the peroxidase enzyme abundantly expressed in neutrophil granulocytes, converts chlorophenols to chlorinated dioxins and dibenzofurans, a human biosynthesis of dioxins from ubiquitous chlorophenols is possible (Wittsiepe et al., 2000).

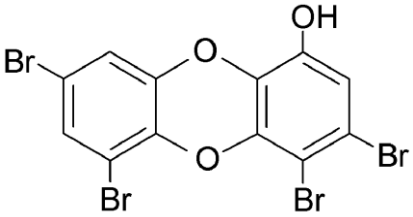


**FIG. 1: SPONGIADIOXIN A (1-HYDROXY-3,4,6,8-TETRABROMODIBENZO [1,4] DIOXIN) PRODUCED BY THE AUSTRALIAN SPONGE *DYSIDEA DENDYI* AS REPELLENT.**

Dioxins are fat-soluble substances, which inevitably accumulate in the food chain. Some of them are toxic [Ballschmitter et al., 1996] and the most toxic is the famous Seveso dioxin, the 2,3,7,8-tetra-chloro-dibenzo-para-dioxin (TCDD), against which toxicity of all other congeners is measured. The toxic congeners include only 29 compounds out of a total of 419 substances (7 PCDDs, 10 PCDFs and 12 PCBs, called PCB-dioxin-like).

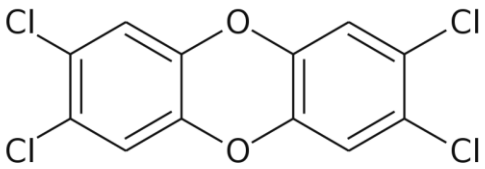


**FIG. 2: 2,3,7,8-TETRACHLORO-DIBENZO-P-DIOXIN (C.A.S. N° 1746-01-6).**

TCDD [CAS N° 01/06/1746] (Fig. 2), was listed by the *International Agency for Research on Cancer* (IARC) among human carcinogens, based on carcinogenicity derived from studies that have combined epidemiological data and mechanistic information obtained in animal-based experiments. These experiments support the ability of TCDD to alter the mechanisms controlling the cell proliferation and growth [IARC, 1997]. Epidemiological studies related to high-dose human exposure and conducted on industrial plant workers, have shown that dioxin exposure involves a linear increase in the ratio of standardized mortality (SMR) for all types of cancer, without any specificity for particular tumours; moreover, such an increase is observed in the case of exposures 100-1000 times higher than that found for general population, only comparable to those generally used in animal studies [Steenland et al., 1999]. However, other epidemiological studies, mainly conducted on subjects occupationally exposed to dioxins and correlated with an increased incidence of either some types of cancer (lung cancer, non-Hodgkin's lymphoma, soft tissue sarcoma) or different non-neoplastic diseases (type 2 diabetes mellitus, ischemic heart disease, chronic respiratory disease, thyroiditis, and thyroid disease), have provided highly controversial results [Bodner et al., 2003, Michalek et al., 2008; Consonni et al., 2008]. Therefore, new epidemiological investigations based on larger collections of enrolled individuals and higher homogeneity of the different compared subgroups are needed, in order to establish the real role of dioxins as human carcinogen [Steenland et al., 2004]. Due to the real difficulties in promoting such epidemiological studies, a series of analyses have been carried out on human and animals tissues, aimed at assessing the cellular and molecular events that occur at this level following exposure to TCDD. This type of studies received a further boost from the recent availability of new proteomic strategies and innovative methodologies, such as confocal microscopy. This latter has been recently used to assess the role of TCDD in the regulation of growth and reproductive development by interfering with the hypothalamic system [Clements et al., 2009].

Dioxins are byproducts of combustion processes and, in the past, were present as unwanted elements in the production of substances such as: DDT (insecticide), 2,4 D (herbicide), 2,4,5 T (defoliant), 2.4,5 TCP (wood preservative), hexachlorophene (disinfectant), PVC (plastic) and other compounds. Currently, the main source of dioxins is represented by the combustion of municipal and hospital waste. They are produced when the combustion process of materials containing chlorine occurs in the absence of oxygen and at temperatures below 800°C.

Incineration plants have been requiring for a long time technical devices which guarantee the permanence of the flue gas at a temperature not lower than 850°C and for a time sufficiently long, so as to ensure a complete destruction of all the products of the incomplete combustion, including precisely dioxins (these latter are also removed by adsorption or catalytic oxidation systems). Although the total amounts are therefore greatly reduced, the combustion of waste still produces dioxins; a recent study has established that 70% - 90% of the total dioxins produced by such combustion is constituted by PCDF, about 20% is represented by PCDD, while the PCBs are found at very low levels [Shibamoto et al., 2007].

In relation to emission sources, the approximate percentages of dioxins annually produced in Europe are: incineration of municipal waste, 28%; domestic heating (wood, coal, etc.), 18%; hospital waste incinerators, 17%; foundries and industrial combustion, 16%; fires and illegally burning waste, 11%; transport vehicle, 2%; other 8%. [USEPA, 1995; HMIP, 1995; Quaß et al., 2004].

In recent years, in response to legal restrictions, a considerable reduction of emissions of dioxins regarding incinerators and industrial sources has been recorded, but an equally satisfactory result for the other non-industrial sources has not been reached yet [Quaß et al., 2004]. Assuming that industrial sources will always be better controlled and consistently contained or reduced, we can hypothesize that in the next future, the non-industrial sources will have a predominant role in the environmental dioxins emission.

The most important route of human exposure to dioxins is food, due to the bioaccumulation of these contaminants in the food chain. Food of animal origin normally contributes to about 80% of total exposure and, in general, more than 90% of human exposure to dioxin comes from food and especially from dietary fat (Fig. 3). In fact, dioxins are extremely lipophilic, with remarkable solubility and stability in the fatty tissues of different organisms, and might be accumulated, even thousands of times, along the food chain, particularly in high fatty concentration foods (milk, cheese, butter, meat, eggs, edible oils, etc.).

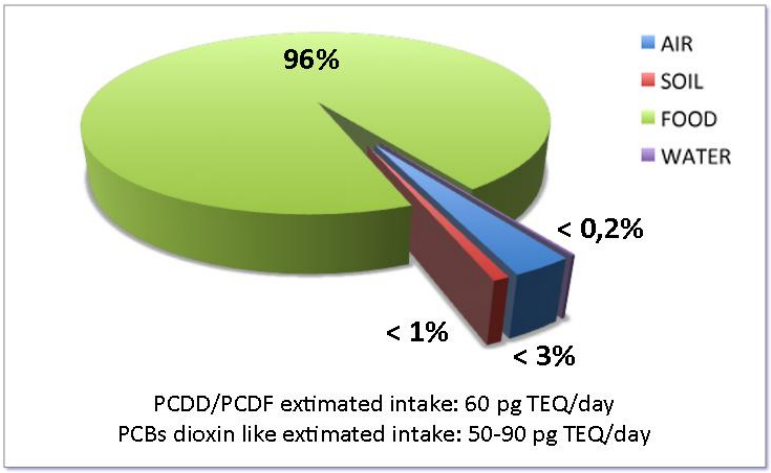


**FIG. 3: SOURCES OF DIOXIN THAT CONTRIBUTE TO HUMAN EXPOSURE. FOOD ACCOUNTS FOR ABOUT 96% OF HUMAN EXPOSURE TO DIOXIN.**

Reduction of human exposure to dioxins necessarily passes through the control of animal feed (the amount of dioxins in animals is directly related to feed contamination) and through the identification of the major emission sources in the area. Only in this way appropriate policies, aimed at reducing contamination of animal feed and long-term emissions can be planned and implemented.

## III. RECEPTOR ACTIVATION MECHANISM OF ARYL HYDROCARBONS (AHR)

To date, the mechanism of action of dioxins and dioxin-like PCBs is believed to begin from the interaction of these compounds with the aryl hydrocarbon receptor (AhR) towards which TCDD presents the highest affinity (in humans and rodents) compared to the other congeners (KD - TCDD ≈ 7 x $10^{-12}$ M) [Brandfield et al., 1998; Denison et al., 2003]. This fact could explain its greater toxicity.

AhR is a ubiquitous cytosolic protein, highly conserved during evolution, which belongs to the family of transcription factors "basic helix-loop-helix/Per-Arnt-Sim (bHLH / PAS)", which are characterized by different functional domains (Fig. 4). The bHLH motif is localized in the N-terminal region of the protein and is common in transcription factors. The nuclear localization signal (NLS) sequence, which allows the transport of the protein inside the nucleus through the nuclear pore complex (NPC), is also present in the N-terminal region. The protein transport to the nucleus is carried out by the intervention of import or nuclear transport cytosolic receptors, called importins, that, in the case of AhR, are the importin-α and the importin-β as well as a small monomeric GTPase, called Ran. Like all the G proteins, Ran is active when bound to GTP and inactive when bound to GDP. In the cytosol Ran is complexed with a protein called GAP (GTPase Activator Protein), an enzyme that inactivates Ran, promoting its GTPase activity (ie stimulating Ran to hydrolyze GTP to GDP + Pi). In the nucleoplasm instead, Ran is complexed with the protein GEF (Guanilic nucleotide Exchange Factor) that activates Ran, causing it to exchange GDP with GTP. This means that a gradient of two conformational forms of Ran (Ran-GTP and Ran-GDP) can be created between inside and outside of the nucleus, which drives the transport in the appropriate direction.

AhR functional domains, distinctive and highly conserved in this superfamily of receptors, are located: i) in the basic region; (b) in the region containing the DNA binding domain; ii) in the HLH region, which contains the helix-loop-helix motif, necessary for protein-protein interactions; iii) in two regions (PAS and PAS-A-B) presenting the dimerization and ligand-binding domains, which specifically interact with other proteins containing PAS domains [Coumailleau et al., 1995; Goryo et al., 2007] or share a high homology with the domains of the following proteins: Per (period circadian protein), Sim (single-minded protein) and ARNT (aryl hydrocarbon receptor nuclear translocator protein) [Ema et al., 1992]; iv) in the glutamine-rich C-terminal region, which contains the transactivation domain for the recruitment of co-activators [Kumar et al., 2001] (Fig. 4).

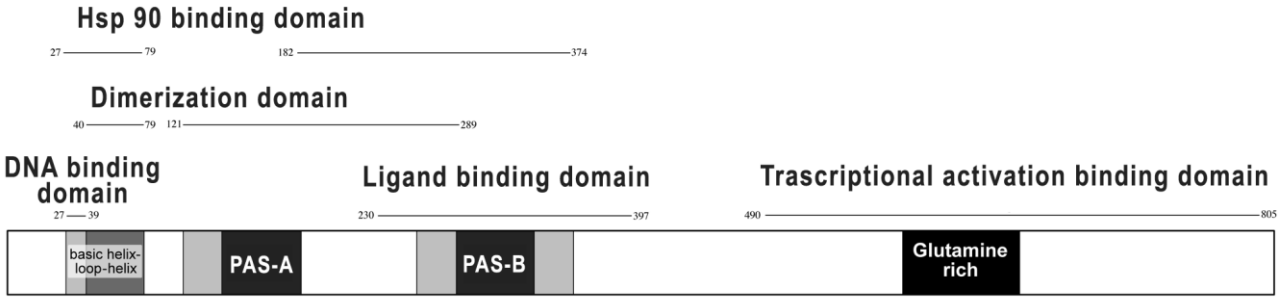


**FIG. 4. ARYL HYDROCARBON RECEPTOR (AHR) FUNCTIONAL DOMAINS.**

In humans, the AhR gene is located on chromosome 7 and consists of 12 exons that encode for a protein of 848 amino acids with a nominal weight of 96,147 Dalton [Le Beau et al., 1994; Ema et al, 1994; http:/ / www.uniprot.org/uniprot/P35869]. It is located in an inactive form in the cytosol, where it forms a multi-protein complex with a dimer of the chaperone HSP90 (90-kDa Heath Shock Protein) [Denis et al., 1988; Perdew et al., 1988], the co-chaperone P23 (tubulin binding protein) [Cox et al., 2004; Kazlauskas et al., 1999], and a subunit of XAP-2 (hepatitis B virus X-associated protein or immunophilinlike protein), also referred to as AIP or ARA9 [Meyer et al., 1998].

The assembly of this multimeric complex takes place in several stages. Initially, there is an immature complex AhR-HSP90 which is stabilized by the intervention of P23. The latent complex AhR-HSP90-P23 is formed and the ligand (TCDD), which enters the cell by simple diffusion due to its high lipophilicity [Gu et al., 2000], binds to the receptor pocket of the PAS-B domain of AhR. The integrity of the resulting complex ligand-AhR-HSP90-P23 is the pre-requisite for the recruitment of XAP-2, whose function is to redistribute the complex in the cytoplasmic compartment of the cell. Subsequent to the formation of the ligand binding, HSP90 induces a conformational change in the N-terminal portion of AhR, which makes accessible its NLS sequence to the importin-α [Kazlauskas et al., 2001] (Fig 5). In the presence of a HSP90 inhibitor, the geldanamycin (GA), the ligand-receptor binding is prevented: this demonstrates the complex role that HSP90 plays in regulating the intracellular traffic of AhR [Kazlauskas et al., 2001].

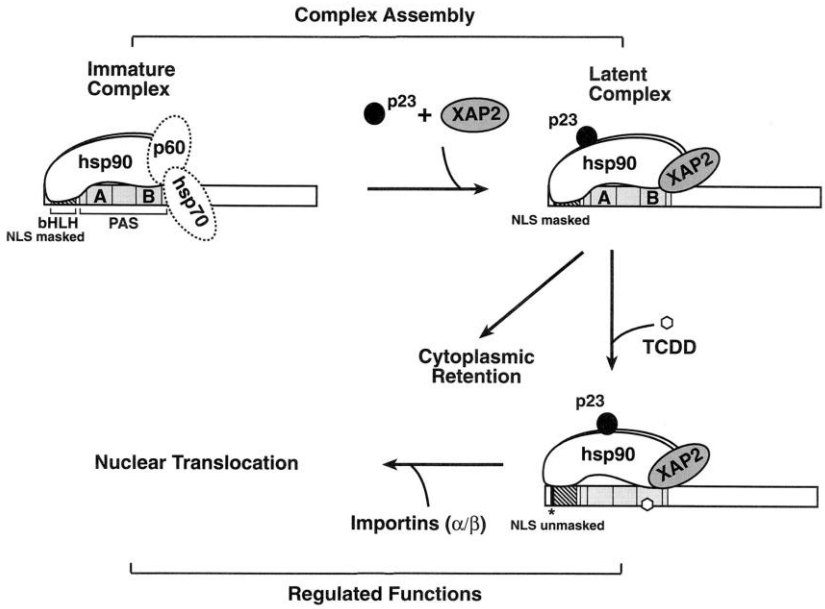


**FIG. 5. MODEL OF AHR MULTIMERIC COMPLEX FORMATION AND OF ITS INTERACTION WITH THE LIGAND DIOXIN.**

The importin-α, in turn, performs the function of acceptor of importin-β that mediates the passage of the whole protein complex through the NPC. In fact, the dissociation of HSP90 from the multimeric complex ligand-receptor does not take place before the entire complex translocates to the nucleus [Heid et al., 2000] (Fig. 5). In the nucleus, AhR dimerizes with its translocation factor ARNT [Reyeset al., 1992; Probst et al, 1993] and the dimerization induces the HSP90 release from the

complex [McGuire et al., 1994; Kazlauskas et al., 1999] (Fig. 6). The heterodimer AhR/ARNT binds to specific regions on the DNA known as Dioxins Response Elements (DRE) or Xenobiotics Response Elements (XRE) [Matsushita et al., 1993; Watson et al., 1992] located in the 5' region of the AhR-responsive genes. The DRE/XRE are regulatory elements placed upstream of the transcription start site of dioxin-inducible genes including, in example, those encoding for enzymes which metabolize xenobiotics (such as CYP1A1, CYP1A2 and glutathione S-transferase [Poland et al., 1982]). The binding of the AhR/ARNT heterodimer on the DRE/XRE elements involves the destruction of the nucleosome, the recruitment of transcription factors on the promoter region, and the subsequent mRNA synthesis.

Currently, AhR and ARNT seem to directly bind to the general transcription factors. Indeed, in mice it has been shown that AhR/ARNT interact with the Transcription Factor IIF (TFIIF), while in humans AhR/ARNT interacts with both TFIIF and the transcription factor that specifically binds to sequences of DNA known as "TATA Box" (TATA-binding protein, TBP), which also represents the binding site of RNA polymerase II (Pol II or RNAP II) [Rowlands et al, 1996; Beischlag et al., 2002]. ARNT has been shown to also interact with other co-activators, such as SRC1, p300/CBP, RIP140 and p160 [Rowlands et al, 1996; Beischlag et al., 2002; Elferink et al., 1990], which in turn mediate the destruction of the nucleosome through their histone acetyl-transferase (HAT) activity (Fig. 6).

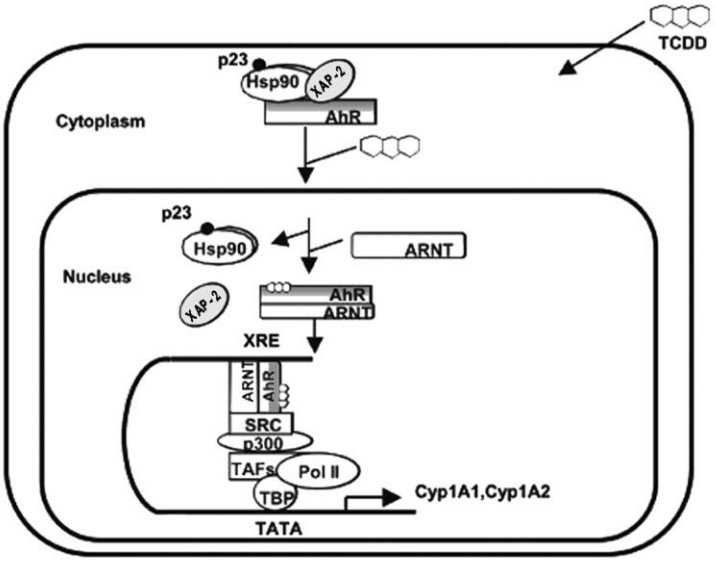


**FIG. 6. TRASCRIPTIONAL ACTIVATION MEDIATED BY AHR-TCDD.**

It is important to underline that in the different animal species the mechanisms of signal transduction (starting from the transcriptional activation mediated by AhR-TCDD/ligand), are not exactly the same and that also this diversity affects the biological and toxicological interspecies responses. Therefore, it is worth remembering that:

- SRC1, coactivator 1 of the steroid receptor (also known as NCOA1 or coactivator of nuclear receptor 1), is a protein endowed with intrinsic histone acetyltransferase activity and its main function is to assist and support the activation of specific DNA sequences, once they have been bound by the estrogen receptor (ER).
- In the family of coactivators p300/CBP, the p300 protein (also known as EP300 or E1A p300 binding protein), characterized by a HAT activity, is important in the processes of cell proliferation and differentiation. In many tumours, mutations in EP300 gene have been observed at somatic level (i.e. acquired during lifespan) in some cancer types only (colorectal, stomach, pancreas, breast, and prostate). In the prostate cancer, the presence of p300 mutated forms allows to predict the rate of the tumour growth and the metastatic spread of the disease.
- RIP140 or Nuclear Receptor-Interacting Protein 1 (NRIP1) is another nuclear protein that modulates the transcriptional activity of a variety of factors (including the estrogen receptor) in the heart, skeletal-muscle, and liver tissues, which has an important role in regulating the metabolism of lipids and glucose.

The molecular details of the transcriptional activation events exemplified in Figure 6 are not definitively elucidated. In fact, new alternatives to the basic mechanism mentioned above have been described, as a result of the isolation of new coactivators with special properties, such as the coiled-coil coactivator (CoCoA) [Kim et al, 1996], and the complex with

many subunits called Mediator [Wang et al, 2004], isolated in both mice and humans.

Once the heterodimer AhR/ARNT with the entire battery of coactivators binds the regulatory gene regions containing the sequences XRE, the corresponding genes are transcriptionally activated and the synthesis of proteins with different functions begins. Among them, there are different enzymes that metabolize xenobiotics (mostly, from the cytochrome P450 system, such as CYP1A1) [Hoffer et al, 1996; Watson et al., 1992] as well as the AP-1 protein and the proto-oncogene products (c-Fos and c-Jun), which act in turn as well-known transcriptional regulators [Puga et al, 1992; Suh et al., 2002].

## IV. BIOLOGICAL AND TOXICOLOGICAL RESPONSES TO THE DIOXINS EXPOSURE

The superfamily of the proteins cytochrome P450 (P450) is involved in oxidative metabolism and elimination of a large number of xenobiotics, including many carcinogens, pro-carcinogens and anticancer drugs. In some cases, however, the detoxification processes mediated by P450 can lead to the formation of intermediates more toxic than the starting compounds, as it happens for benzo[a]pyrene, which is bio-activate by these enzymes in an toxic carcinogen intermediate. The members of the CYP1 family of P450 are AhR-inducible (Fig. 6). In particular, dioxins modulate the expression of CYP1A1 and CYP1B1 [Watson et al, 1992; Whitlock et al., 1999]. In addition, dioxins also modulate the expression of other P450 enzymes, such as CYP2S1 [Rivera et al, 2002], which contributes to the metabolism of environmental carcinogens through a NADPH independent activity.

Many xenobiotic compounds, such as polycyclic aromatic hydrocarbons (PAHs), although less strong AhR ligands compared to TCDD, also modulate the expression of CYP1A1 and CYP1B1 through the same mechanism described for dioxins and are detoxified by the same enzymes that they have contributed to express. This is not the case for dioxins, which are resistant to biotransformation and accumulate without any modification in the lipid matrix of the body with long half-lives (in men, 7.5 years for TCDD). This means that dioxins cannot trigger a mechanism of carcinogenesis similar to that of many environmental carcinogens, such as benzo[a]pyrene, and of many endogenous substances, such as estrogen. The 17-β estradiol (E2), in fact, is firstly converted to 4-hydroxy-estradiol by a specific 4-hydroxylase (CYP1B1) and then into a reactive semiquinone/quinone intermediate [Liehr et al, 2000]. This reactive intermediate is able to form adducts with DNA, starting with that sequence of events that leads to breast cancer, as it has been demonstrated *in vivo* in rats [Li et al, 2004] (Fig. 7). Exposure to high doses of dioxins, while stimulates the metabolism of E2, leading to an increased risk of breast cancer, also produces the inhibition of the expression of some E2-inducible genes. This has led to hypothesize the existence of a cross-talk between AhR and ERα (estrogen alpha receptor), which has not sufficiently clarified yet [Matthews et al, 2006].

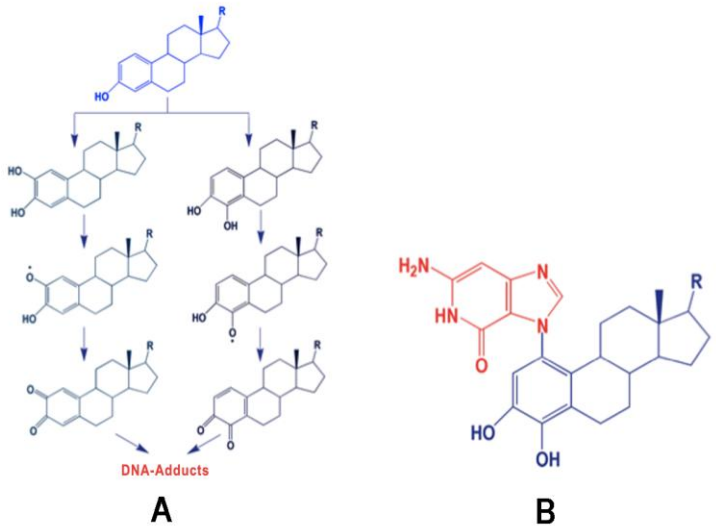


**FIG. 7. BIOTRANSFORMATION OF ESTROGEN $E_2$ AND DNA ADDUCT FORMATION. THE PROPOSAL MECHANISM FOR THE HORMONAL CARCINOGENESIS INVOLVES BOTH PROLIFERATIVE EFFECTS OF ESTROGENS AND GENOTOXIC EFFECT OF THEIR METABOLITES. THE LATTER EFFECT IS ATTRIBUTED TO THE OXIDATION OF CATHECOLESTROGENS TO SEMI-QUINONES AND QUINONES AND FORMATION OF REACTIVE OXYGEN SPECIES THAT IN TURN REACT WITH DNA (A) FORMING ADDUCTS WITH N7-GUANINE (B).**

The evidence of the existence of a regulatory cross-talk between estrogen and dioxin was firstly highlighted in 1978 [Kociba et al, 1978], in a study based on Sprague-Dawley rats fed for two years with TCDD in quantities of 0,001, 0.01 and 0.1 μg/kg/day, equivalent to 2,200, 210, and 22 parts per trillion (ppt) of TCDD. This study demonstrated the existence of the toxic and carcinogenic effects of TCDD in female rats treated with high dose levels (2,200 ppt), which was highly attenuated at average dose levels (210 ppt). On the other hand, the same study showed that low dose level exposure (22 ppt) did not exert toxic effects but, as well, produced an unexpected protective action for breast and uterus cancers in female rats. The data by Kociba et al. have been successively rearranged in 1985 by the EPA, as tumours in specific organs for a range of doses [http://www.epa.gov/ne/ge/thesite/restofriver/reports/final_hhra/comments/generalelectric/AttachM.pdf]. It was pointed out that, by correlating the exposure dose with the tumour frequency and considering the total number of tumours in all tissues of female rats, a U shaped dose-response curve can be obtained (Fig. 8). A low risk for liver cancer could be also encountered at even low and intermediate doses of exposure [http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=300025QF.txt].

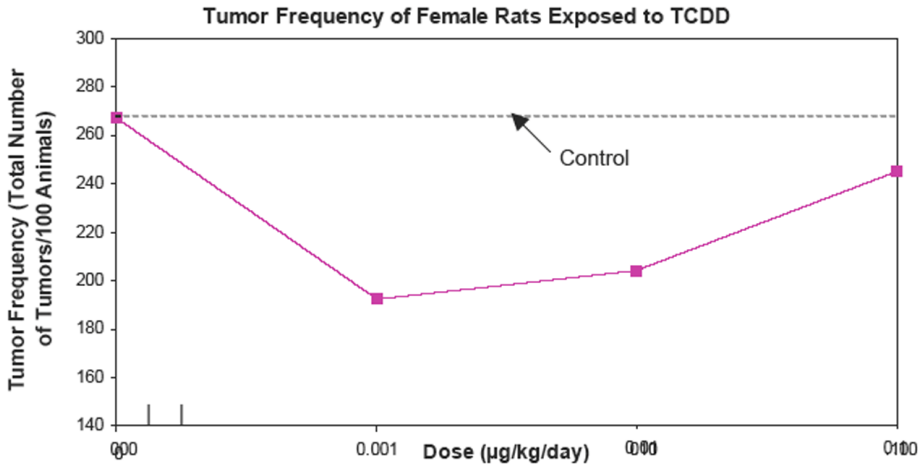


**FIG. 8. DOSE-RESPONSE RELATIONSHIP FOR RAT FEMALE EXPOSURE TO TCDD.**

Based on their findings, Kociba et al. were able to establish that 1 ng/kg/day of TCDD represented "the no observable adverse effect level" (NOAEL). From this study, finally emerged that low doses of TCDD in rats may act as a potent anticarcinogen.

The study by Kociba et al. represented a cornerstone during all these years and has been replicated several times in the light of new acquisitions in this field. A revision made in 1991 [Keenan et al, 1991] had moved the NOAEL for the rat liver cancer from 0,001 μg/kg/day to 0.01 g/kg/day and, thanks to the new criteria of assessment and calculating methods for predicting the response to human exposure, an estimated 16-time lower risk of exposure to TCDD has been determined.

More recently, the paper by Kociba et al. has been used for a patent application (Patent No. 6,444,698) for the TCDD use in the treatment of cancer in both men and women [Kayajanian et al, 2003]. In fact, based on a critical reading of existing studies on the TCDD toxicity and evidence that the TCDD half-life in humans is approximately 7.5 years (conversely, TCDD does not accumulate in the rat), it has been highlighted that TCDD could be considered as:

- a sort of "promoter blocker" for some cancers, that IARC had instead indicated to be promoted by dioxins;
- a promoter of other cancers not classified as dioxin-induced by the EPA;
- a net anticarcinogen [Kayajanian et al, 1997].
- Based on the observation that in the study by Kociba et al., TCDD inhibits, in a dose-dependent manner, the formation and/or the growth of hormone-dependent breast and uterus cancers, other studies had already been conducted aimed to show that dioxins play a chemopreventive and chemotherapeutic action in breast cancers [Holcomb et al, 1994; Tritscher et al., 1995] and that it is possible to develop new chemotherapeutic agents, called SAhRM (Selective Aryl hydrocarbon Receptor Modulators), for a combined treatment of breast cancer. In particular, such new compounds should be characterized by the presence of the same anti-oestrogenic activity of the dioxin and absence of the toxic responses mediated by AhR. The prototype of these compounds was represented by the 6-Methyl-1,3,8-trichlorodibenzofuran, which inhibits the growth of breast cancers in female Sprague-Dawley

rats at doses lower than 50 μg/kg/day [Safe et al, 1999 ].

Recently, a study that further supports the role played by TCDD in inhibiting the growth of breast cancer has been published [Barhoover et al, 2010]. In this paper it has been shown that AhR, without activation by exogenous ligands, may functionally interact with CDK4, resulting in cell cycle regulation for both estrogen responsive (ER+) and estrogen non-responsive (ER-) breast cancer cell lines. This molecular interaction assigns AhR a role as molecular switch during the cell cycle, further confirming the previous model indicating that AhR may promote the cell cycle progression. The new integrated model explains, for example, the lack of the breast development in AhR-knockout mice. In the presence of low doses of exogenous ligands, such as TCDD or even SAhRM, the AhR activation changes the polarity of the molecular switch and automatically the cell cycle is induced to stop. This new integrated model explains both the epidemiological data [Bertazzi et al, 1997] and the results obtained on rodents in which AhR exogenous ligands induced the inhibition of breast tumorigenesis.

The large amount of data accumulated over the years has shown that at high dose levels of exposure, dioxins cause a plethora of adverse effects ranging from chloracne to development impairments, from thymus atrophy and immunological problems to cancer. Conversely, other data, though less extensively, have shown that at low dose levels of dioxins play a beneficial effect. Dioxins, therefore, do not seem to escape the Paracelsus' axiom that says: "*Omnia sunt venenum: nec sine venenum quicquam existit. Dosis sola facit ut venenum not fit*" (All things are poison, and nothing is without poison; only the dose permits something not to be poisonous). In addition, research studies seems to increasingly support the concept that there is a dioxins amount, a "low dose" of exposure, not yet defined, which performs essential biological functions for the normal activities of the animal and human cell. However, when this "low dose" of exposure is exceeded, dioxins toxic effects take place, as with any other substance. Despite the fact that this effect has been already seen by Kociba et al. since 1978, as reported in Figure 8, great resistance has been done in the world of science to accept that the dose-response relationship for exposure at low dose levels to dioxins had not a linear trend (as it happens with the high dose level), but a U shaped pattern, which is said hormetic. This resistance results from the statement that the hormetic trend is used as a key to the interpretation of homeopathy. However, the hormesis is a widespread natural phenomenon according to which chemical, biological substances or physical agents carry out helpful activities in living organisms at low exposure levels, while become toxic or dangerous to higher levels of exposure. The dose-response curve, as it is known, is not unique to all substances that exhibit hormetic activity. In fact, the hormetic response can be:

- directly induced (adaptive response with bio-positive effects that translate dynamic and stimulatory events);
- due to over-compensating for changes in homeostasis

Despite the scepticism and resistance from the past, recently the concept of hormesis has begun to penetrate the traditional fortress of occupational and environmental toxicology, as in Germany [Lutz, 2000], Finland [Tuomisto et al, 2005] and USA [Zhang et al, 2009] and it is finding its disciplinary structure also on theoretical bases [Calabrese, 2003; Calabrese, 2008]. (72-73). Of course, as usually happens during radical changes, the question concerning the carcinogenic risk at low dose levels of exposure and about the trend of the dose-response curve, whether it is linear or nonlinear and whether or not it shows the existence of a threshold and/or of a hormetic effect, is far from being answered [Walker et al, 2007]. Further studies are awaited, also by adopting new investigation approaches, as those offered by omics-sciences and by the new vision of "systems biology ', which have begun to give definitive answers to similar questions in relation to ionizing radiations [Zhao et al, 2010].

In fact, the hypothesis about the U shape for the dose-response curves in the case of low doses exposure to ionizing radiations and of the beneficial effect played by ionizing radiations in the case of low dose levels exposure, for their ability to stimulate protective systems of the cell, is now finally confirmed. This result, as it has been pointed out, will involve the necessity to review all the laws relating to protective measures for the general and public health at the workplace [Zhao et al, 2010].

Meanwhile, researches, even the traditional ones, are going on in order to identifying new biological responses to dioxins exposure, as it has happened with the discovery of ecto-ATPase as a novel protein expressed in response to dioxin exposure. [Gao et al, 1998]. This is a ubiquitous, trans-membrane and calcium-and magnesium-dependent enzyme of eukaryotic cells which, catalyzing the hydrolysis of extra-cellular ATP into ADP and inorganic phosphorus, plays a role in signal transduction induced by ATP/ADP [Plesner et al, 1995]. These activities will help to get further knowledge necessary to obtain more insight into the biological functions induced by the low level of dioxin dose exposure in balancing to the already known toxicological functions occurring at high level of dose exposure.

## ACKNOWLEDGEMENTS

Authors are grateful to Prof. Dr. Gabriele Sabbioni and Prof. Corrado Lodovico Galli for the thoughtful revision of the manuscript.

## REFERENCES

[1] Ballschmitter, K., and Bacher, R., *Dioxine*, VCH Verlag Weinheim, (1996) ISBN 3-527-28768-X.
[2] Barhoover, M.A., Hall, J.M., Greenlee, W.F., and Thomas, R.S., (2010). Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4. *Mol. Pharmacol.* **77,** 195-201.
[3] Beischlag, T.V., Wang, S., Rose, D.W., Torchia, J., Reisz-Porszasz, S., Muhammad, K., Nelson, W. E., Probst, M.R., Rosenfeld, M.G., and Hankinson, O., (2002). Recruitment of the NCoA/SRC-1/p160 family of transcriptional coactivators by the Aryl Hydrocarbon Receptor/Aryl Hydrocarbon Receptor Nuclear Translocator complex. *Mol. Cell. Biol*. **22,** 4319-4333.
[4] Bertazzi, P.A., Bernucci, I., Brambilla, G., Consonni, D., Pesatori, A.C., (1998). The Seveso studies on early and long-term effects of dioxin exposure: A review. *Environ. Health Perspectives.*, **106,** 625-633.
[5] Bertazzi, P.A., Zocchetti, C., Guercilena, S., Consonni, D., Tironi, A., Landi, M.T., and Pesatori, A.C., (1997). Dioxin exposure and cancer risk: a 15-year mortality study after the "Seveso accident". *Epidemiology*. **8,** 646-652.
[6] Bodner, K.M., Collins, J.J., and Bloemen, L.J., (2003). Cancer risk for chemical workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Occup Environ Med*.**60,** 672-675.
[7] Brandfield, C.A., and Poland, A., (1988). A competitive binding essay for 2,3,7,8-tetrachloro-dibenzo-p-dioxin and related ligands of the Ah receptor. *Mol. Pharmacol.* **34,** 682-688.
[8] Calabrese, E.J., (2008). Hormesis: Principles and Applications for Pharmacology and Toxicology. *Am. J. Pharmacol. Toxicol.* **3,** 59-71.
[9] Calabrese, E.J., and Baldwin, L.A., (2003). HORMESIS: The Dose-Response Revolution. *Annu. Rev. Pharmacol. Toxicol.* **43,** 175-197.
[10] Clements, R.J., Lawrence, R.C., and Blank, J.L., (2009). Effects of intrauterine 2,3,7,8-tetrachlorodibenzo-p-dioxin on the development and function of the gonadotrophin releasing hormone neuronal system in the male rat. *Reprod. Toxicol.* **28,** 38-45.
[11] Consonni, D., Pesatori, A.C., and Zocchetti, C., (2008). Mortality in a population exposed to dioxin after the Seveso, Italy, accident in 1976: 25 years of follow-up. *Am. J. Epidemiol.* **167,** 847-858.
[12] Coumailleau, P., Poellinger, L., Gustafsson, J.A., and Whitelaw, M.L., (1995), Definition of a minimal domain of the dioxin receptor that is associated with Hsp90 and maintains wild type ligand binding affinity and specificity. *J. Biol. Chem.*; **270,** 25291-300.
[13] Cox, M.B., and Miller, C.A., (2004). Cooperation of heat shock protein 90 and p23 in aryl hydrocarbon receptor signaling. *Cell Stress Chaperones.* **9,** 4-20. Anche Cox MB, Miller CA. 2002. The p23 co-chaperone facilitates dioxin receptor signaling in a yeast model system. Toxicol Lett 129: 13–21.
[14] Denis, M., Cuthill, S., Wikström, A.C., Poellinger, L., and Gustafsson, J.A, (1988). Association of the dioxin receptor with the Mr 90,000 heat shock protein: a structural kinship with the glucocorticoid receptor. *Biochem. Biophys. Res. Commun.* **155,** 801-817.
[15] Denison, M.S., Nagy, S.R., (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu. Rev. Pharmacol. Toxicol*. **43**, 309-334.
[16] Elferink, C.J., and Whitlock, J.P. Jr., (1990). 2,3,7,8-Tetrachlorodibenzo-p-dioxin-inducible, Ah receptor-mediated bending of enhancer DNA. *J. Biol. Chem*. **265,** 5718-5721.
[17] Ema, M., Matsushita, N., Sogawa, K., Ariyama, T., Inazawa, J., Nemoto, T., Ota, M., Oshimura, M and Fujii-Kuriyama Y, (1994). Human arylhydrocarbon receptor: functional expression and chromosomal assignment to 7p21. *J. Biochem.* **116,** 845-851.
[18] Ema, M., Sogawa, K., Watanabe, N., Chujoh, Y., Matsushita, N., Gotoh, O., Funae, Y., and Fujii-Kuriyama, Y., (1992). cDNA cloning and structure of mouse putative Ah receptor. *Biochem. Biophys. Res. Commun*. **184,** 246-253.
[19] Fernandez-Salguero, P.M., Ward, J.M., Sundberg, J.P., and Gonzalez, F.J., (1997). Lesions of aryl-hydrocarbon receptor-deficient mice. *Vet. Pathol.*; **34,** 605-614.
[20] Gao, L., Dong, L., and Whitlock, J.P.Jr., (1998). A novel response to dioxin. Induction of ecto-ATPase gene expression. *J. Biol. Chem*. **273,** 15358-15365.
[21] Goryo, K., Suzuki, A., Del Carpio, C.A., Siizaki, K., Kuriyama, E., Mikami, Y., Kinoshita, K., Yasumoto, K., Rannug, A., Miyamoto, A., Fujii-Kuriyama, Y., and Sogawa, K., (2007). Identification of amino acid residues in the Ah receptor involved in ligand binding. *Biochem. Biophys. Res. Commun*.; **354,** 396-402.
[22] Gu, Y. Z., Hogenesch, J. B., and Bradfield, C. A., (2000). The PAS superfamily: sensors of environmental and developmental signals. *Ann. Rev. Pharmacol. Toxicol*. **40,** 519-561.
[23] Heid, S.E., Pollenz, R.S., and Swanson, H.I., (2000). Role of heat shock protein 90 in mediating agonist-induced activation of the aryl hydrocarbon receptor. *Molecular pharmacology.* **57,** 82-92.
[24] Her Majesties Inspectorate of Pollution (HMIP). '*A Review of Dioxin Emissions in the UK'*, Report No. DOE/HMIP/RR/95/004, Department of the Environment, 1995
[25] Hoffer, A., Chang, C.Y., and Puga, A., (1996). Dioxin induces transcription of fos and jun genes by Ah receptor-dependent and -independent pathways. *Toxicol. Appl. Pharmacol*. **141,** 238-247.
[26] Holcomb, M., and Safe, S., (1994). Inhibition of 7,12-dimethylbenzanthracene-induced rat mammary tumor growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Cancer Lett*. **82,** 43-47.
[27] Horii, Y., van Bavel, B., Kannan, K., Petrick, G., Nachtigall, K., and Yamashita, N., (2008). Novel evidence for natural formation of dioxins in ball clay. *Chemosphere*. **70,** 1280-1289.
[28] http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=300025QF.txt
[29] http://www.epa.gov/ne/ge/thesite/restofriver/reports/final_hhra/comments/generalelectric/AttachM.pdf
[30] http://www.uniprot.org/uniprot/P35869

[31] IARC-International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Polychlorinated Dibenzo-para-dioxins and Polychlorinated Dibenzofurans.* Vol. 69. 666 pp. Lyon, France: IARC, 1997.
[32] JECFA. 2001. Joint FAO/WHO Expert Committee on Food Additivies, fifty-seventh meeting, Rome, 5-14 June and Schecter, A., Birnbaum, L., Ryan, J.J., Constable, J.D., (2006). Dioxins: an overview. *Environ Res.*;**101,** 419-428.
[33] Kayajanian, G., (1997). Dioxin is a promoter blocker, a promoter, and a net anticarcinogen. *Regul. Toxicol. Pharmacol*. **26,** 134-137.
[34] Kayajanian, G., (2003). Clinical trials of TCDD. *Nonlinearity in Biology, Toxicology, and Medicine*. **1,** 149-153.
[35] Kazlauskas, A, Poellinger, L., and Pongratz, I, (1999). Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. *J. Biol. Chem*. **274**(19), 13519-13524
[36] Kazlauskas, A., Sundstrom, S., Poellinger, L., and Pongratz, I., (2001). The HSP90 chaperone complex regulates intracellular localization of the dioxin receptor. *Mol. Cell. Biol.* **21,** 2594-2607.
[37] Keenan, R.E., Paustenbach, D.J., Wenning, R.J., and Parsons, A.H., (1991). Pathology reevaluation of the Kociba et al. (1978) bioassay of 2,3,7,8-TCDD: Implications for risk assessment. *J. Toxicol. Environ. Health, Part A: Current Issues*. 1991; **34,** 279-296.
[38] Kim, J.H., and Stallcup, M.R., (2004). Role of the coiled-coil coactivator (CoCoA) in aryl hydrocarbon receptor-mediated transcription. *J. Biol. Chem*. **279,** 49842-49848.
[39] Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. Proc Natl Acad Sci USA 2008; 105:9721–6.
[40] Kociba, R.J., Keyes, D.G., Beger, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.L., Barnard, S.D., Hummel, R.A., and Humiston, C.G., (1978). Results of a 2-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. *Toxicol. Appl. Pharmacol*. **46,** 279-303.
[41] Kumar, M.B., Ramadoss, P., Reen, K.R., Vanden Heuvel, J.P., and Perdew, G.H., (2001). The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity. *J. Biol. Chem.* **276,** 42302-42310.
[42] Le Beau, M.M., Carver, L.A., Espinosa, R., Schmidt, J.V., and Bradfleld, C.A.,(1994). Chromosomal localization of the human AHR locus encoding the structural gene for the Ah receptor to 7p21-pl5. *Cytogenet. Cell Genet*. **66,** 72-176.
[43] Li, K.-M., Todorovic, R., Devanesan, P., Higginbotham, S., Kofeler, H., Ramanathan, R., Gross, M.L., Rogan, E.G., and Cavalieri E.L., (2004). Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. *Carcinogenesis.* **25,** 289-297.
[44] Liehr J.G., (2000). Is Estradiol a Genotoxic Mutagenic Carcinogen? Endocrine Reviews **21,** 40–54.
[45] Lutz, W.K., (2000). A true threshold dose in chemical carcinogenesis cannot be defined for a population, irrespective of the mode of action: Commentary. *Biological effects of low level exposures* (*Belle*). **9,** 1,6 (http://www.belleonline.com/newsletters/volume9/vol9-1/n6v91.html)
[46] Matsushita, N., Sogawa, K., Ema, M., Yoshida, A., and Fujii-Kuriyama, Y., (1993). A factor binding to the Xenobiotic Responsive Element (XRE) of P-4501A1 gene consists of at least two Helix-Loop-Helix proteins, Ah Receptor and Arnt. *J. Biol. Chem*. **268,** 21002-21006.
[47] Matthews, J., and Gustafsson, J-Ä., (2006). Estrogen receptor and aryl hydrocarbon receptor signaling pathways. *Nuclear Receptor Signaling.* **4,** e016.
[48] McGuire, J., Whitelaw, M.L., Pongratz, I., Gustafsson, J.A., and Poellinger, L., (1994). A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor. *Mol. Cell. Biol.* **14,** 2438-2446.
[49] Meyer, B.K., Pray-Grant, M.G., Vanden Heuvel, J.P., and Perdew, G.H., (1998). Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. *Mol. Cell. Biol.* **18,** 978-988.
[50] Michalek, J.E., and Pavuk, M., (2008). Diabetes and cancer in veterans of Operation Ranch Hand after adjustment for calendar period, days of spraying, and time spent in Southeast Asia. *J. Occup. Environ. Med.* **50,** 330-340.
[51] Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T.N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., and Fujii-Kuriyama, Y., (1997). Loss of teratogenic response to 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells.*, **2,** 645-654.
[52] Perdew, G.H., (1988). Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* **263,** 13802–13805.
[53] Plesner, L., (1995). Ecto-ATPases: identities and functions. *Int. Rev. Cytol.* **158,** 141-214.
[54] Poland, A., and Knutson, C., (1982). 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol***. 22,** 517-554.
[55] Probst, M.R., Reisz-Porszasz, S., Agbunag, R.V., Ong, M-S., and Hankinson, O., (1993). Role of the Aryl Hydrocarbon Receptor Nuclear Trans Locator Protein in Aryl Hydrocarbon (Dioxin) Receptor Action. *Mol. Pharmacol.* **44,** 511-518.
[56] Puga, A., Nebert, D.W., and Carrier, F., (1992). Dioxin induces expression of c-fos and c-jun proto-oncogenes and a large increase in transcription factor AP-1. *DNA Cell Biol.* **11,** 269-281.
[57] Quaß, U., Fermann, M., and Broker, G., (2004). The European Dioxin Air Emission Inventory Project-Final Results. *Chemosphere.* **54,** 1319-1327.
[58] Quintana FJ, Basso AS, Iglesias AH. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 2008; 453:65–71.
[59] Reyes, H., Reisz-Porszasz, S., and Hankinson, O., (1992). Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science.* **256,** 1193-1195.
[60] Rivera, S.P., Saarikoski, S.T., and Hankinson, O., (2002). Identification of a novel dioxin-inducible cytochrome P450. *Mol. Pharmacol*. **61,** 255-259.
[61] Rowlands, J.C ., McEwan, I.J., and Gustafsson, J.A., (1996). Trans-activation by the human aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator proteins: direct interactions with basal transcription factors. *Mol. Pharmacol*. **50,** 538-548.
[62] Safe, S., Qin, C., and McDougal, A., (1999). Development of selective aryl hydrocarbon receptor modulators (SARMs) for treatment of breast cancer. *Expert Opin. Invest. Drugs*. **8,** 1385-1396.
[63] Schmidt, J.V., Su, G.H., Reddy, J.K., Simon, M.C., and Bradfield, C.A., (1996). Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc. Natl. Acad. Sci. USA*; **93,** 6731-6736.
[64] Shibamoto, T., Yasuhara, A., and Katami, T., (2007). Dioxin formation from waste incineration. *Rev. Environ. Contam. Toxicol.* **190,**

1-41.
[65] Sorg, O., Zennegg, M., Schmid, P., Fedosyuk, R., Valikhnovskyi, R., Gaide, O., Kniazevych, V., Saurat, J-H., (2009). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. *Lancet.* **374**,1179-1185.
[66] Steenland, K., Bertazzi, P., and Baccarelli, A., (2004). Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen. *Environ. Health Perspect.* **112,** 1265-1268.
[67] Steenland, K., Piacitelli, L., Deddens, J., Fingerhut, M., and Chang, L. I., (1999). Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Natl. Cancer Inst.* **91,** 779-786.
[68] Stevens EA, Mezrich JD, Bradfield CA. The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. Immunology 2009; 127: 299–311
[69] Stockinger B., (2009). Beyond toxicity: aryl hydrocarbon receptor-mediated functions in the immune system. *J. Biology*., **8**, 61.
[70] Suh, J., Jeon, Y.J., Kim, H.M., Kang, J.S., Kaminski, N.E., and Yang, K.H., (2002). Aryl hydrocarbon receptor-dependent inhibition of AP-1 activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in activated B cells. *Toxicol. Appl. Pharmacol.* **181,** 116-123.
[71] Tritscher, A.M., Clark, G.C., Sewall, C., Sills, R.C., Maronpot, R., and Lucier, G.W., (1995). Persistence of TCDD-inducedhepatic cell proliferation and growth of enzyme altered foci after chronic exposure followed by cessation of treatment in DEN initiated female rats. *Carcinogenesis.***16,** 2807-2811.
[72] Tuomisto, J., Pekkanen, J., Kiviranta, H., Tukianen, E., Vartiainen, T., Viluksela, M., and Tuomisto, J.T., (2005). Dioxin cancer risk – Example of hormesis? *Dose-Response*. **3,** 332-341.
[73] USEPA. *Locating and Estimating Air Emissions from Sources of Dioxins and Furans*, Office of Air Quality Planning and Standards, Research Triangle Park, NC, USA, 1995
[74] Walker, N.J., (2007). Toxicological Highlight. Unraveling the Complexities of the Mechanism of Action of Dioxins. *Toxicol. Sci.* **95,** 297-299.
[75] Wang, S., Ge, K., Roeder, R.G., and Hankinson, O., (2004). Role of Mediator in Transcriptional Activation by the Aryl Hydrocarbon Receptor. *J. Biol. Chem*. **279,** 13593-13600.
[76] Watson, A.J., and Hankinson, O., (1992). Dioxin- and Ah receptor-dependent protein binding to xenobiotic responsive elements and G-rich DNA studied by in vivo footprinting. *J. Biol. Chem*. **267,** 6874-6878.
[77] Whitlock, J.P. Jr., (1999). Induction of cytochrome P4501A1. *Annu. Rev. Pharmacol. Toxicol*. **39,** 103-125.
[78] Wittsiepe, J., Kullmann Y., Schrey P., Selenka F. and Wilhelm M., (2000). Myeloperoxidase - catalyzed formation of PCDD/F from chlorophenols, *Chemosphere*, **40**, 963-968.
[79] Zhang, Q., Pi, J., Woods, C.G., and Andersen, M.E., (2009). Phase I to II Cross-induction of xenobiotic metabolizing enzymes: a feedforward control mechanism for potential hormetic responses. *Toxicol. Appl. Pharmacol*. **237,** 345-356.
[80] Zhao, Y., and Ricci, P.F., (2010). Modeling dose-response at low dose: a systems biology approach for ionization radiation. *Dose-Response*. **8,** 456-477.