Review

Virologie

Virologie 2018, 22 (1) : E1-E13

Chemical pollution and innate antiviral immunity: Dangerous Liaisons ?

Simon Hayek[1]
Nicolas Pietrancosta[1]
Rodolphe Alves de Sousa[1]
Robert Barouki[2]
Jean-Philippe Herbeuval[1]
Pierre-Olivier Vidalain[1]

[1] Équipe chimie & biologie, modélisation et immunologie pour la thérapie (CBMIT), CNRS UMR8601, Université Paris Descartes, Paris, France
<pierre-olivier.vidalain@parisdescartes.fr>
[2] Toxicologie pharmacologie et signalisation cellulaire, Inserm UMR-S1124, Université Paris Descartes, Paris, France

Abstract. Environmental pollution is of concern to civil society and as the problem intensifies, there is increasing pressure on politicians and polluters to assess and mitigate this risk. In addition, the emergence (or re-emergence) of viral pathologies such as dengue or chikungunya has also become a major concern requiring appropriate measures. Unfortunately, these two issues may well collide with unpredictable consequences in the next decades. Indeed, a growing number of studies suggests that organic pollutants could alter the innate antiviral response, including the type I interferon system (IFN-I). Such interactions could have significant consequences on the susceptibility of populations to viral infections, but also modify responses and protection induced by vaccines or favor the development of autoimmune diseases. The purpose of this review is to take stock of the known interactions between organic pollutants and the IFN-I response, and to present questions that should be addressed in the future in order to better assess this risk.

Key words : interferons, innate immunity, virus, chemical pollution, pesticides, AhR

Résumé. La pollution environnementale préoccupe la société civile et à mesure que le problème s'amplifie, des pressions de plus en plus fortes s'exercent sur les pouvoirs publics et les pollueurs pour évaluer et limiter ce risque. En outre, l'émergence (ou la réémergence) de pathologies virales tels que la dengue ou le chikungunya est également devenue un sujet d'inquiétude nécessitant la mise en place de mesures appropriées. Malheureusement, ces deux problématiques pourraient bien trouver un point de convergence inattendu dans les décennies à venir. En effet, un nombre croissant d'études suggère que des polluants organiques pourraient altérer la réponse immunitaire innée antivirale, et notamment la réponse interféron de type I (IFN-I). Étant donné l'importance physiologique de celle-ci, de telles interactions pourraient avoir des conséquences non négligeables sur la sensibilité des populations aux infections virales, mais aussi modifier notre réponse à certains vaccins ou favoriser le développement de maladies auto-immunes. L'objet de cette synthèse bibliographique est donc de dresser un bilan des interactions connues entre pollutions chimiques et réponse IFN-I, et de présenter certaines pistes qui mériteraient d'être explorées dans le futur pour mieux appréhender ce risque.

Mots clés : interférons, immunité innée, virus, pollution chimique, pesticides, AhR

Introduction

In 2005, Dr. Christopher Wild proposed the concept of "exposome", a concept that encompasses all the environmental factors to which an organism is exposed from conception to death: chemical molecules in the environment, food, radiations, social interactions, etc. All of these factors combine with inherited genetic and epigenetic determinants to shape an organism's development over the course of life. This concept is particularly important in epidemiology and for human health because the triggering of many diseases such as cancer is directly related to

Reprints : P.-O. Vidalain

doi:10.1684/vir.2018.0717

To cite this article: Hayek S, Pietrancosta N, Alves de Sousa R, Barouki R, Herbeuval JP, Vidalain PO. Chemical pollution and innate antiviral immunity: Dangerous Liaisons ? *Virologie* 2018; 22(1):1-13 doi:10.1684/vir.2018.0717

environmental factors. Chemical pollutants are an essential component of the exposome, and the word "toxome" has been quoined to encompass all these toxic molecules [1]. Indeed, we would be exposed to more than 80,000 natural or artificial chemicals whose potential effects on the body are largely unknown. To overcome this shortcoming, several projects have recently been launched in Europe, the United States and Asia to study globally and using high-throughput methods the potential effects of the toxome on different cellular functions [1].

Studies that evaluate the impact of the toxome on the body focus primarily on the carcinogenic, mutagenic and reprotoxic ("CMR") potential of chemical substances. This is the basis of international classification on hazardous chemicals for health that is applied to manufactured goods, household products, food additives and working environment for example. Most surprisingly, the impact of chemical substances on immunity is not systematically tested, which is nonsense since the primary function of the immune system is precisely to detect external aggressions to orchestrate an appropriate response. Nevertheless, the idea that chemical pollutants influence the immune response and the development of viral infections in particular is not new, and several studies have supported this hypothesis in both animals and humans. For example, insecticides in the neonicotinoid class have demonstrated immunosuppressive effects in bees at doses well below the lethal dose. These molecules thus increase the susceptibility of insects to viral infections, which would significantly contribute to the collapse of bee populations observed since the early 1990s and the marketing of neonicotinoids [2]. Many chemical pollutants also have a proven impact on the immune system of marine mammals [3]. Because of their position at the top of the food chain and their adaptation to the marine environment, these animals accumulate certain pollutants in their fats, which gives them a role as sentinels of this ecosystem. By inducing a state of immunosuppression, chemical pollutants such as heavy metals or dioxins could promote the spread of certain infectious agents, including the incidence of certain morbilliviruses that regularly decimate seal and cetacean populations. Finally, in humans, it has been established that air pollution by nitrogen dioxide (NO2), ozone (O3), fine particles or polycyclic aromatic compounds very significantly increases the susceptibility to infections by respiratory viral diseases and the severity of associated symptoms [4]. Several studies have notably established a strong link between the level of air pollution and the number of hospitalizations related to respiratory viral infections.

Chemical pollutants such as dioxins can affect innate and adaptive immune responses, and impact virtually all the cell populations involved: epithelial cells, monocytes, dendritic cells, neutrophils, T and B lymphocytes, NK cells, etc. [5]. Some of these molecules would in particular alter an essential component of the innate antiviral immunity: the type I interferon response (IFN-I). IFN-I are cytokines produced by most cells when infected by a virus (or simply in contact with a virus for some of them), and which induce a local and/or global state of resistance to viruses by inducing a large set of antiviral genes (*figure 1*). IFN-I also play a key role in triggering and/or maintaining many autoimmune and inflammatory conditions such as systemic lupus erythematosus, type I diabetes, polymyositis or Sjogren's Syndrome. By altering the IFN-I response, chemical pollutants may have underestimated effects on susceptibility to viral infections and the onset of autoimmune and inflammatory conditions. The purpose of this bibliographic synthesis is to take stock of the known interactions between chemical pollution and the IFN-I response, and to present some hypothesis that deserve to be explored in the future.

Interferons and the antiviral innate immune response

IFN-I is composed of a family of cytokines that can be subdivided into five groups: IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω [6]. IFN-α and β are the most studied because of their predominant role in the antiviral innate immune response. Each of these IFN-I is encoded by a single gene, with the exception of IFN-α which are produced from 13 paralogous genes. IFN-I bind to their membrane receptor, IFNAR1/2c, resulting in the phosphorylation of STAT1 and STAT2 transcription factors by the tyrosine kinases JAK1 and TYK2 (*figure 1*). Once phosphorylated, STAT1 and STAT2 assemble into homo or heterodimers that, in combination with IRF9 for STAT1/2 and STAT2/2 dimers, activate more than 300 target genes: the ISGs (Interferon-Stimulated Genes). The products of these different genes, such as MxA or Tetherin, inhibit viruses at specific steps of their replication cycle, thus giving to IFN-I exceptional antiviral properties. IFN-I also play an essential role at the interface between innate and adaptive immune responses, these cytokines effectively contributing to the activation, selection and proliferation of T and B lymphocytes specific for an infectious agent.

The production of IFN-I is triggered by Pathogen-Associated Molecular Patterns or PAMPs, and molecular patterns associated with tissue damage or DAMPs (Damage-Associated Molecular Patterns). These PAMPs and DAMPs correspond to molecular structures present specifically in certain lipids, sugars, nucleic acids or proteins, and signal the presence of a pathogen or damaged cells in the microenvironment [7]. They are therefore "danger signals" for the cell. Different classes of cellular receptors, called PRRs (Pattern Recognition Receptors), participate in the recognition of PAMPs and DAMPs. The main PRRs

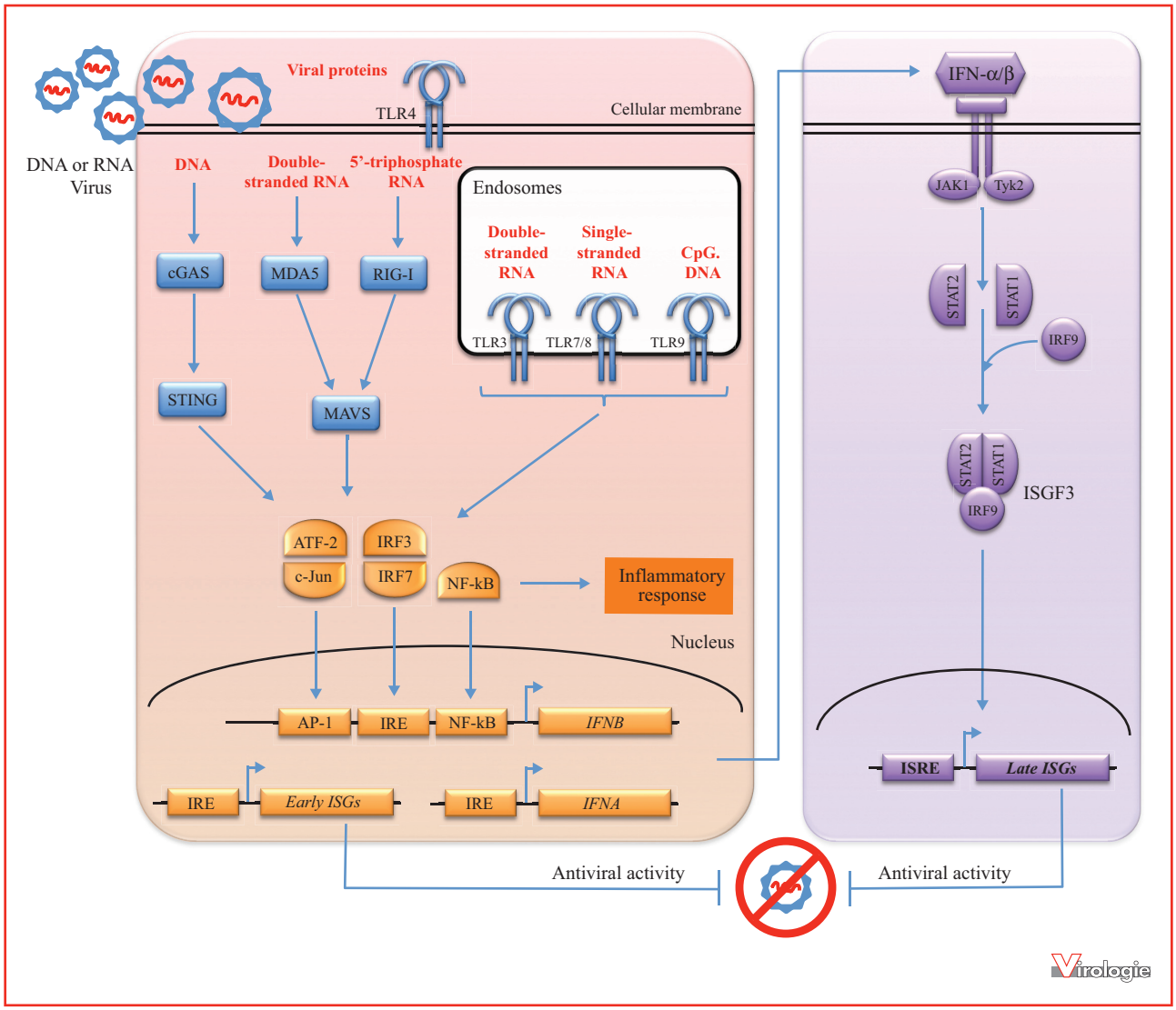

Figure 1. Schematic of the IFN-I response. When a virus is contacting a cell, membrane receptors (TLR2/3/4/7/8/9) and cytosolic receptors (cGAS, MDA5, RIG-I) are activated by envelope glycoproteins of the virus or nucleic acids present in the viral particle. The signaling cascades downstream of these receptors converge towards a set of genes encoding antiviral cellular factors, the early Interferon-Stimulated Genes (ISGs), and induce IFN-I in parallel. These cytokines act through autocrine and paracrine signaling to activate the transcription factors STAT1, STAT2 and IRF9, and induce a second wave of antiviral genes, the late ISGs. Meanwhile, the engagement of PRRs leads, *via* NF-κB and AP-1 signaling, to the expression of pro-inflammatory factors.

involved in inducing the IFN-I response are Toll-Like Receptors 2, 3, 4, 7, 8 and 9 (TLR2/3/4/7/8/9), the cytosolic RIG-Like Receptors (RLR) which recognize viral RNAs, and a heterogeneous set of cytosolic DNA sensors such as cGAS or IFI16 (*figure 1*). With the exception of TLR2 and 4, the PRRs involved in the antiviral response essentially recognize nucleic acids. Once engaged, they activate signaling cascades that all converge towards NF-κB, AP-1 and the transcription factors of the IRF family ("Interferon Regulatory Factors"), in particular IRF3 and IRF7. While NF-κB and AP-1 primarily control the inflammatory response, IRF3 and 7 bind to promoters of some ISGs to induce an early antiviral response. In parallel, they also activate the transcription of IFN-I genes, alone or by associating with NF-κB and AP-1 in the case of IFN-β. Once secreted, IFN-I activate a second wave of ISGs, *via* the IFNAR1/2c receptor and the JAK/STAT signaling cascade, to enhance the innate antiviral response in both infected and non-infected neighboring cells. Moreover, IFN-I secretion induces the expression of chemokines allowing the

recruitment and activation of leucocytes involved in both innate and adaptive immune responses.

Toll-like Receptors or TLRs play a central role in the induction of IFN-I [7]. They are transmembrane receptors whose extracellular domain allows the recognition of PAMPs and DAMPs, while the intracellular domain recruits different adapters for signal transduction, including Myd88 for TLR2/4/7/8/9, and TRIF for TLR3/4 and potentially TLR2. These adapters then allow the activation of ubiquitin ligases and kinases, in particular TBK1 and IKK-ε, which lead to the activation of IRF3/5/7. Besides detecting pathogens found outside the cells or internalized in endosomes, TLRs are also involved in the detection of intracellular viruses or bacteria present in the cytosol through autophagy mechanisms. Indeed, autophagy allows the transfer of cytosolic elements to the lumen of endosomes where the recognition domains of TLRs are localized. Among the major TLRs involved in the antiviral response, TLR3 is expressed by conventional dendritic cells, or cDCs, macrophages as well as epithelial and endothelial cells of certain tissues, especially in the respiratory tract. It is inserted in the plasma or endosomal membranes depending on cell type and activation state, and recognizes double-stranded RNAs. TLR7, 8 and 9 receptors are mainly localized in the endosomal pathways. While TLR7 and 8 recognize single-stranded RNAs, TLR9 is a specific receptor for DNA with CpG motifs. While TLR8 is strongly present in monocytes, the expression of TLR7 and 9 is more specific for lymphoid cells, especially B cells and plasmacytoid dendritic cells or pDCs. Although pDCs represent less than 1% of peripheral blood leukocytes, they play a key role in the production of IFN-I. Once activated, they produce 1,000 to 10,000 times more IFN-α than other cells. As such, they are referred to as IPC for "Interferon Producing Cells" [8]. Finally, and as mentioned above, TLR2 and 4 can induce the expression of IFN-I. However, and in contrast to other TLRs involved in IFN-I induction, they do not recognize nucleic acids but different components of bacteria, fungus and parasite membranes or cell walls as well as endogenous ligands such as heat shock proteins (HSPs). In addition, TLR2 and 4 respond to certain viral proteins, including envelope glycoproteins. As such, these two TLRs seem to play a significant role in the response to certain viral infections. They are notably expressed by the myeloid cells (peripheral blood monocytes, tissue-resident macrophages, microglial cells, etc.), granulocytes, and epithelial and endothelial cells of different tissues.

RLRs correspond to the second major family of PRRs involved in virus recognition, and comprises three members: MDA5, which recognizes large double-stranded RNAs, RIG-I that activates in the presence of double-stranded RNAs with a 5′ bi- or tri-phosphate extremity (3P-RNA), and finally LGP2 that regulates the activation of RIG-I. Once activated, MDA5 and RIG-I bind to the MAVS adapter present at the mitochondrial membrane, which then recruits the TBK1/IKK-ε kinases to achieve IRF3 phosphorylation. These receptors are exclusively cytosolic and are therefore involved in the recognition of viral RNAs within infected cells.

Finally, many intracellular receptors, such as IFI16, LRRFIP1, DHX9, DHX36 or DDX41, detect the presence of DNA in the cytosol, which is a danger signal characteristic of DNA virus infections but also a marker of energetic or genotoxic stress. The enzyme cGAS and the adapter STING form a particularly remarkable system. The cGAS protein has a DNA binding domain that triggers its activation and the synthesis of a cyclic dinucleotide, cGAMP. This molecule then plays the role of secondary messenger by binding to STING that, once activated, recruits TBK1 and induces the synthesis of IFN-I. Unlike TLRs whose expression is limited to peripheral blood leukocytes and some tissues that are entry points for pathogens, cytosolic receptors for RNA and DNA are expressed by the vast majority of cells to allow a rapid local response to infections or tissue damage. Finally, it is important to note that IFN-I increase the expression of many PRRs, thus promoting the detection of infectious agents and the activation of the innate immune response.

Dioxins and the AhR Receptor

Of the chemical pollutants that are under careful surveillance, polychlorinated dibenzo-*p*-dioxins (PCDDs or simply dioxins) have a proven impact on health and the environment. These extremely toxic molecules are produced by the accidental combustion (or uncontrolled incineration) of materials containing chlorine, but also when manufacturing some herbicides or plastics. The most toxic of these molecules is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or TCDD (*figure 2*). Present in the Orange Agent used as a defoliant during the Vietnam War, dioxins were also the cause of the Seveso disaster in 1976. Very lipophilic, they accumulate in the food chain and persist in the body during years. These compounds are thus classified as major persistent organic pollutants (POPs). In addition to their acute toxicity, they could impact the reproductive system over several generations [9] and have been associated with the development of cancers [10] and certain malformations [11], but this last point is disputed. In addition, dioxins are important disrupters of innate and adaptive immune responses, and their immunosuppressive effects are fairly well documented [12, 13].

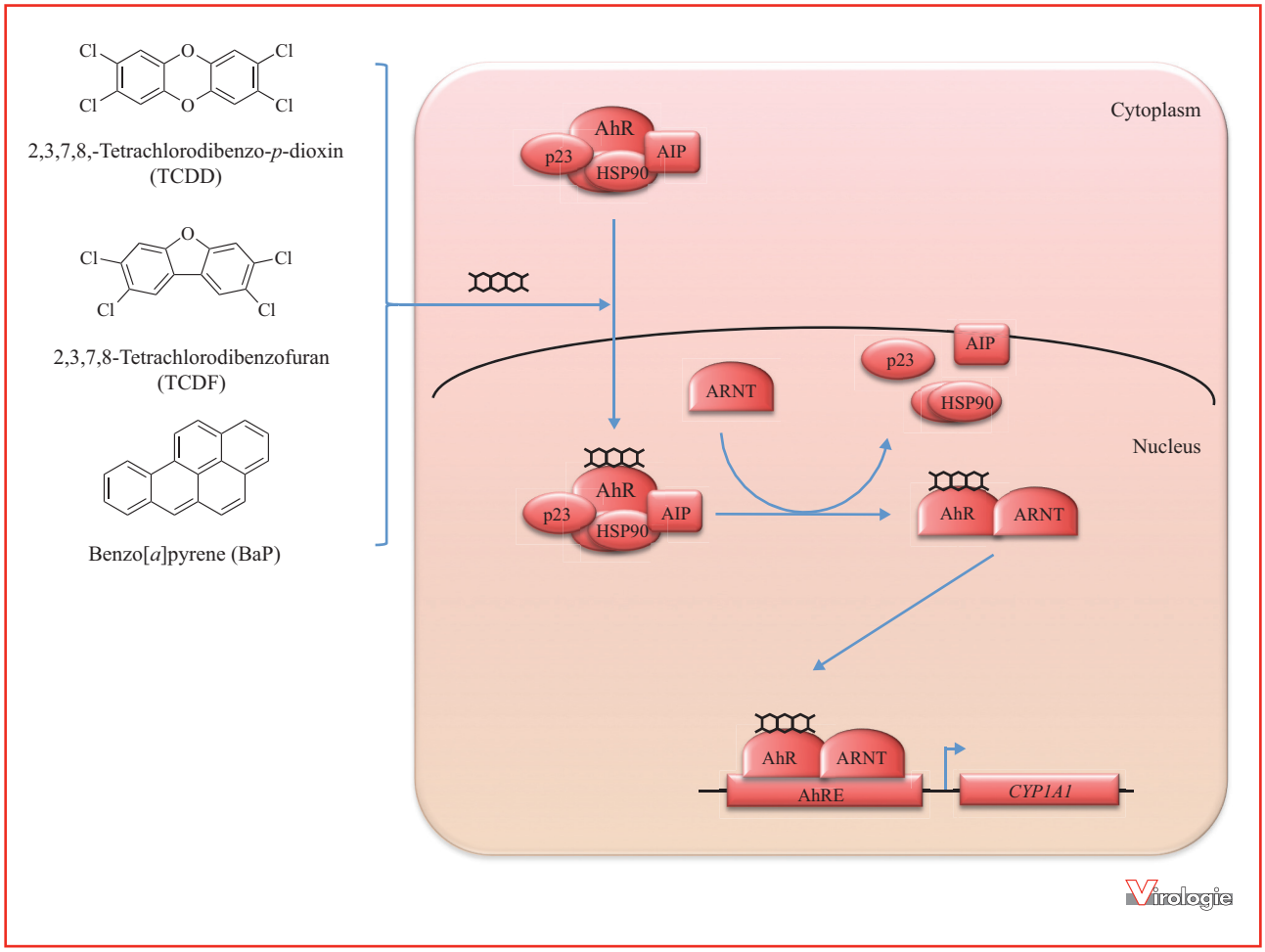

Figure 2. Schematic of AhR activation by organic pollutants. In the absence of ligand, the AhR protein is sequestered in the cytosol where it binds p23, AIP and HSP90. In the presence of TCDD, TCDF or BaP, AhR dissociates from the p23, AIP and HSP90 proteins, either in the nucleus or cytoplasm (doubts remain on this point), and undergoes translocation in the nucleus where it binds to the ARNT protein. Then, the AhR-ARNT complex binds to the AhR response elements (AhRE), and induces the transcription of target genes such as CYP1A1.

Numerous studies that aimed at identifying the mechanisms responsible for dioxin activity in the body have led to the identification of the AhR receptor (Aryl Hydrocarbon Receptor) [12, 13]. This transcription factor belongs to the superfamily bHLH/PAS (basic helix-loop-helix/Per-Arnt-Sim). In normal conditions, AhR is associated with HSP90, the co-chaperone protein p23, and AIP (AhR Interacting Protein), which altogether maintain AhR in an inactive state and prevent its degradation by ubiquitination (*figure 2*). The binding of TCDD to AhR causes a conformational change that unmasks a nuclear localization signal and triggers AhR translocation to the nucleus. Then, AhR associates to the cofactor ARNT (AhR Nuclear Translocator) and binds to the AhR Response Element (AhRE) that is present in the promoter of many genes, in particular detoxification factors such as cytochromes P450 (CYP1A1, CYP1A2 and CYP1B). In addition to TCDD and other polychlorinated dibenzo-*p*-dioxins, AhR is a receptor for a large number of pollutants, including halogenated aromatic hydrocarbons such as polychlorinated dibenzofurans (PCDFs), including 2,3,7,8- tetrachlorodibenzofuran (TCDF, *figure 2*), and polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (BaP, *figure 2*). These pollutants are not manufactured on purpose, but are produced by incomplete combustion of petroleum derivatives or charcoal for BaP, or chlorinated plastics such as PVC for PCDFs. It should be noted that AhR is also activated by various exogenous natural compounds from diet (quercetin, indol-3-carbinol, etc.), as well as endogenous molecules, in particular derivatives of tryptophan such as 6-formylindolo[3,2-b] carbazole (FICZ),

kynurenine, bilirubin or oxidized short-chain fatty acids such as lipoxin A4. As such, AhR is not only playing a key role in the cellular response to xenobiotics, but also in a large number of endogenous regulatory mechanisms, which would in fact be its primary function [14, 15]. In particular, the study of AhR-/- mice has shown that the activation of AhR by endogenous ligands such as kynurenine participates in the negative feedback of innate and adaptive immune responses [12, 13]. It has also been demonstrated that kynurenin produced by activated pDCs has a tolerogenic and immunosuppressive action, in particular by transforming the helper T lymphocytes into regulatory T cells [16].

Several interactions between AhR and transcription factors having a key role in the IFN-I response have been highlighted. In particular, it has been shown that AhR interacts and regulates the activity of STAT1 and NF-κB transcription factors [17, 18]. In LPS-stimulated macrophages *via* TLR4, AhR associates directly with STAT1 to inhibit NF-κB, thereby blocking IL-6 production and inflammatory response [18]. In the same cells, AhR also binds to the Sp1 transcription factor to suppress induction of histidine decarboxylase in response to LPS, thereby inhibiting the synthesis of histamine and IL-6 [19]. In addition to NF-κB, Sp1, and STATs, AhR also interferes with the TBK1/IRF3 signaling pathway, resulting in a blocking of IFN-β production in response to different viral PAMPs [20]. Indeed, in AhR-/- mouse fibroblasts, the phosphorylation of TBK1 and IRF3 as well as the production of IFN-β are greatly increased in response to various viral infections or activators of the MAVS and STING pathways. In the same cells, the replication of vesicular stomatitis or influenza viruses is reduced. Comparable results were obtained by treating wild-type mouse fibroblasts with a specific AhR inhibitor, CH-223191, or with IDO inhibitors (indoleamine 2,3-dioxygenase), the enzyme that catalyzes the synthesis of kynurenine from tryptophan [20]. Similarly *in vivo*, synthesis of IFN-β in response to the influenza virus is increased, and viral titers reduced, in AhR-/- mice compared to wild-type mice [20]. Identical results were obtained by inhibiting AhR with CH-223191 [20]. These results demonstrate the role of negative regulator exerted by AhR on the innate immune antiviral response, and the role played by its different endogenous ligands. TIPARP, a member of the poly(ADP-ribose) polymerase (PARP) family, is one of the factors induced by AhR. This protein would play a key role in the downregulation of the antiviral innate immune response by inactivating TBK1 by ADP-ribosylation [20].

In vivo, it has been established that dioxins, and in particular TCDD, increase the morbidity and mortality associated with different viral infections [5]. Even at extremely low doses, ranging from 0.01 to 10 µg/kg [21, 22], TCDD altered the immune response to influenza virus in mice and significantly increased animal mortality, although the observed effects vary greatly depending on the experimental protocol and the mouse strain [5]. Similarly, TCDD-exposed mice show increased sensitivity to coxsackievirus B3 [23]. *In vitro*, this organic pollutant also increased HIV replication in different cell lines [5]. TCDD was found to stimulate, among other things, the production of TNF-α and NF-κB activation, thus leading to increased transcription of the provirus [24]. Finally, it is established that TCDD or other AhR ligands also increase the replication of human cytomegalovirus, both *in vivo* and *in vitro*, and is capable of reactivating Esptein-Barr virus (EBV) when latent in infected cells [5, 25, 26]. In the case of EBV, direct interactions between AhR and viral factors such as EBNA3 appear to be involved [5]. In mice, mortality associated with herpesvirus II (HSV-II) is increased by weekly injections of TCDD [27]. Finally, TCDD has been shown to increase *in vitro* the replication of BHV-1 (Bovine Herpesvirus 1), a major pathogen for the cattle industry, which appears to have an effect on the prevalence of this infection in herds [5].

In conclusion, the effects of TCDD and dioxins on viral replication are clearly established, at least under experimental conditions, but remain complex. The AhR receptor has several well-identified roles in the immune response and mediates the effects of endogenous compounds such as kynurenine but also pollutants such as TCDD. The effects of TCDD on viral infections can thus be explained by the action of AhR on both the innate and adaptive immune responses, but also by direct or indirect interaction with proteins and/or viral promoter sequences. The specific effects of these pollutants on the innate antiviral response are still poorly documented despite the well-established role of AhR on IFN-I production. Future studies would allow a better evaluation of the impact of dioxins on the IFN-I response, as well as epidemiological consequences.

Endocrine disruptors

Physiologically, it has been shown that sex hormones regulate the innate immune response *via* estrogen receptors (ER) alpha and beta [28]. This is in particular the case for the female hormone 17β-estradiol, or E2, which increases the production of IFN-α and TNF-α in pDCs in response to TLR7, 8 or 9 ligands [29]. Endocrine disruptors are chemical substances that, by binding to ER-α/β receptors as agonists or antagonists, disrupt the functioning of this hormonal system. Some of these molecules would also act indirectly on estrogen receptors *via* AhR in particular, as physical and functional interactions have been

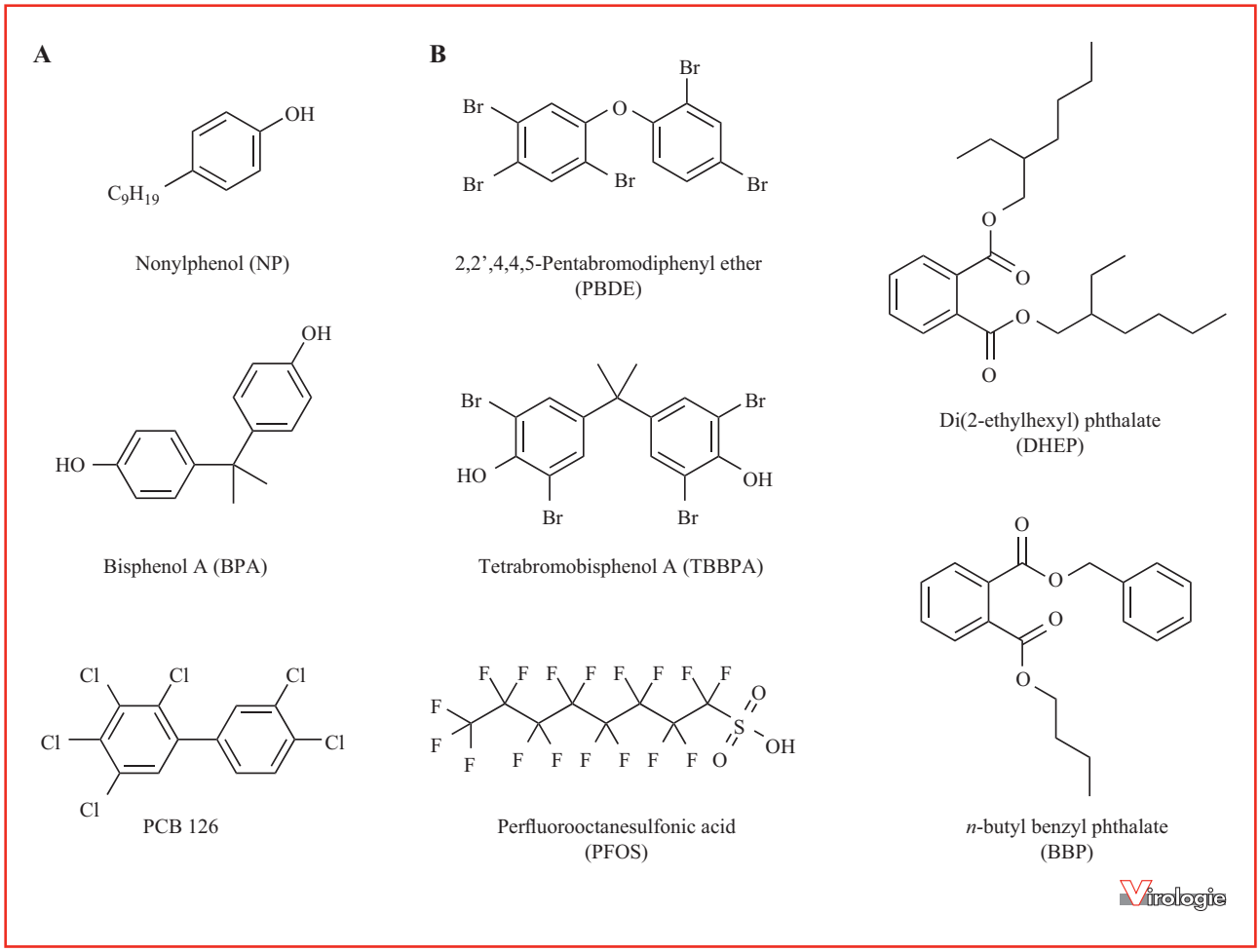

Figure 3. Structures of organic pollutants present in daily materials. (A) Nonylphenol, bisphenol A (PBA) and PCB 126 are known endocrine disruptors. (B) Structures of other pollutants used as flame retardants (PBDE, TBBPA), waterproofing agents (PFOS) or hardeners in plastics (phthalates).

demonstrated between these different factors [12, 13]. Endocrine disruptors have deleterious effects on body function, especially on reproduction, metabolism, the nervous system and defense mechanisms against cancer. Several studies also showed an effect of endocrine disruptors on the immune response, and more particularly on the IFN-I response with cell-type specific effects. Among these organic pollutants are the alkylphenols, which are widely used in the manufacture of many household products (detergents, lubricants, resins, perfumes, flame retardants, etc.). Some of these compounds (or byproducts of their degradation), such as nonylphenol (NP) and 4-octylphenol (4-OP), have estrogenomimetic properties (*figure 3*). In pDCs, NP and 4-OP increase TNF-α production, and at the same time suppress IL-10 and IFN-I production both *in vitro* and *in vivo* [30]. The effects of NP and 4-OP on pDCs partially depend on estrogen receptors, but AhR or the NR3C4 androgen receptor may also be involved [31].

Bisphenol A (BPA) is used as a hardener in the manufacture of plastics (*figure 3*). It has been shown that this molecule induces the expression of different genes of innate immunity at nanomolar concentrations, including IFN-β, in monocytes and macrophages purified from mouse bone marrow [32]. The binding of BPA to the ER-α receptor is responsible for this phenomenon. In these cells, as well as in the THP-1 cell line or primary human monocytes, BPA also induces not only STAT1 activation, but also the expression of NLRP3, a key component of the inflammasome [32]. Since this complex has a central role in the production of pro-inflammatory cytokines, BPA potentially increases the cellular response to pro-inflammatory NLRP3 ligands. It has also been shown *in vivo* in mice that perinatal exposure to BPA increases the permeability of the intestinal

epithelium in adulthood, and alters its immunological functions, thus promoting inflammatory responses [33]. On the other hand, in animals that have undergone the same perinatal treatment, the inflammatory response induced in the lungs by influenza virus infection seems to be inhibited, but without measurable consequences on viral load or animal survival [34]. Mice similarly exposed to BPA also had their response to Theiler virus disturbed, with an increased inflammatory response and exacerbated neurological symptoms in this animal model of multiple sclerosis [35]. Finally, it has been shown *in vitro* that BPA, like 17β-estradiol, inhibits the replication of influenza virus in primary nasal epithelial cells [36]. However, this effect, which is limited to cells of female origin and depends on ER-β receptor expression, is most likely based on an inhibition of cellular metabolism rather than a modulation of the antiviral innate response.

Polychlorinated biphenyls (PCBs) are another class of endocrine disruptors (*figure 3*). They have been massively used as electrical insulators in transformers and capacitors, but also as coolant fluids. As early as 1970, PCBs have been shown to increase the mortality of ducks infected with the hepatitis virus [37]. Mice given oral PCBs are also more susceptible to herpes virus 1 (HSV-1), influenza virus, and ectromelia virus, a murine poxvirus [38, 39]. Finally, although several epidemiological studies have shown the immunomodulatory effects of these persistent organic pollutants on aquatic fauna and marine mammals in particular [3], the direct impact of PCBs on innate antiviral immunity and the IFN-I response needs to be documented. Since some PCBs have a mode of action similar to that of TCDD and bind to AhR, similar effects on the innate immune response are expected.

Everyday materials

Brominated flame retardants are used in many furniture and building materials. They are persistent organic pollutants whose usage is highly regulated. Polybrominated diphenyl ethers (PBDEs) are the most common, and consequences on liver function, thyroid, nervous system and reproduction have been shown (*figure 3*). Several studies have also established an impact of these compounds on the innate immune response and the replication of some viruses. *In vitro*, PBDEs were found to increase the production of inflammatory cytokines by human leukocytes stimulated with LPS [40]. In mice infected with coxsackievirus B3, the administration of an oral dose of the flame retardant BDE-99 (20 mg/kg) significantly increased viral load in the liver and inhibited the expression of the MCP1 chemokine [41]. Tetrabromobisphenol A (TBBPA), another flame retardant (*figure 3*), has been shown to increase the replication of human respiratory syncytial virus (hRSV) as well as the inflammatory response in mice treated for one month with this molecule [42]. Although PBDEs have apparently no effect in this model where adult animals are treated, mice that were born from females exposed to PBDEs during gestation replicate hRSV at higher levels than control animals [43]. While it is difficult to extrapolate these results to humans or animals in their natural environment, these studies suggest a potential link between flame retardants and susceptibility to viral infections.

Perfluoroalkyls (PFAS) such as perfluorooctanesulfonic acid (PFOS) or perfluorooctanoic acid (PFOA) are surfactants, widely used for years as waterproofing agents, and also listed as persistent organic pollutants (*figure 3*). Several studies showed immunosuppressive effects in animal models at doses comparable to that of exposed workers. Potential mechanisms of action include peroxisome proliferation, mitochondrial dysfunction and oxidative stress, effects on steroid hormones, including progesterone, estrogen and testosterone, loss of gap junction intercellular communication, and altered thyroid function [44]. In a mouse model of influenza virus infection, exposure to relatively low doses of PFOS (25 μg/kg) for 21 days led to pronounced weight loss and excess mortality in treated animals [45]. Many epidemiological studies also suggest that high PFAS may impact the response to some vaccines such as rubella and mumps and the associated protection [44]. Finally, in children exposed to PFOS *in utero*, a greater susceptibility to various pathogens, including viruses, has been shown [46]. To date, the potential effect of PFAS on the innate antiviral response and production of IFN-I has not been documented.

Phthalates, which are used as additives in various plastic materials, are also endocrine disruptors and their presence in the home environment is correlated with the development of asthma (*figure 3*). They act on different receptors, including PPAR-α and γ ("Peroxisome proliferator-activated receptor") and various hormone receptors such as ER-α/β and NR3C4 (Androgen receptor). Phthalates can influence cytokine production in human PBMCs, with varying stimulatory or inhibitory effects depending on the cytokines studied and the nature of the phthalates tested [47]. More specifically on the innate immune antiviral response, Kuo *et al.* showed that di(2-ethylhexyl) phthalate (DEHP) and *n*-butyl benzyl phthalate (BBP) inhibit the production of IFN-I in TLR9 ligand-stimulated human pDCs [48]. Furthermore, CD4+ T lymphocytes interacting with pDCs treated with DEHP or BBP expressed lower levels of IFN-γ but higher levels of IL-13, and this correspond to a switch from Th1 to Th2 cytokine profiles. All of these effects of phthalates

on the biology of pDCs could sensitize individuals to viral infections while promoting the development of asthma, allergic reactions and chronic inflammatory conditions such as lupus [49].

Pesticides (insecticides, herbicides, fungicides)

Several pesticides, insecticides or herbicides have been shown to act on the innate antiviral immune response (*figure 4*). Igarashi *et al.* tested the effects of numerous organic pollutants, including various pesticides, on the activation of mouse macrophages by TLR2 or TLR4 ligands [50]. Using NF-κB, TNF-α, and NO synthesis as activation markers, they showed a strong inhibition of TLR2 and TLR4 pathways by benomyl and ziram (fungicides of the carbamate and dithiocarbamate families, respectively), as well as chlordecone and Kelthane (organochlorine insecticides). Using a reporter gene, they also documented the inhibitory effects of benomyl, Kelthane, chlordecone, alachlor (an herbicide of the chloroacetamide family) and ziram on the IFN-β promoter [51].

Benomyl belongs to the carbamate family, which includes many insecticides whose mode of action is based on the inhibition of acetylcholinesterase (*figure 4*). Another member of this family of pesticides is carbaryl (1-naphthyl methylcarbamate), a contaminant of both surface water and groundwater. *In vitro*, it blocked NO synthesis and IFN-β production of mouse macrophages stimulated with LPS [52], and promoted the replication of varicella-zoster virus [53]. *In vivo*, adult Xenopus or tadpoles exposed to carbaryl showed an impaired immune response to FV3 ranavirus ("Frog Virus 3"), including decreased levels of IFN-I [54]. In adult Xenopus exposed to carbaryl at larval stage for three weeks, significant increase in viral load and immunosuppression were also observed. *In vitro*, this same molecule inhibits the synthesis of IFN-I in a fish cell line in response to Goldfish Virus-2 infection [55].

Simazine is an herbicide from the triazine family that inhibits photosynthesis. It has been shown to inhibit IFN and TNF-α synthesis in LPS-stimulated macrophages [56]. Atrazine, another herbicide of the triazine family (*figure 4*), also inhibits the immune response of *Xenopus* to FV3. Inhibition of IFN-I and TNF-α synthesis was observed in treated tadpoles, and correlated with increased mortality following FV3 infection [57]. Finally, it has recently been shown that oral administration of pentachlorophenol (PCP) (*figure 4*), a pesticide used in the treatment of various materials such as wood or pulp, induces the expression of many ISGs as well as IFN-I in the liver of treated mice [58]. Several genes involved in the Nrf2-dependent oxidative stress response were also induced, which could reflect PCP degradation by metabolic enzymes into free-radical producing compounds. However, reverse effects have been reported in LPS-treated mouse macrophages where PCP inhibits IFN-β promoter induction. Since PCP is a well-characterized endocrine disruptor, its binding to estrogen receptors may explain this inhibition of the IFN-I response [51].

Conclusion and perspectives

A concordant array of experimental data shows that different families of organic pollutants may act on the IFN-I response. The biological relevance of these effects is likely as these pollutants bind to receptors like AhR and ER-α/β whose effects on the immune system are well established. However, there are only a limited number of studies available and many questions still need to be addressed, suggesting an under-evaluation of this problem. To date, the work carried out is based mainly on cell culture systems and laboratory animals for *in vivo* studies (mice, rats, *Xenopus*). It is therefore difficult to determine whether the effects observed are transposable to humans, domestic animals and wildlife. The animals used as models, and in particular rodents, have indeed metabolic specificities, which can lead to under or overestimate the immunotoxicity of tested molecules. Moreover, and despite real efforts in the design of experimental protocols, it is complex to mimic the effects of a chronic exposure to pollutants for weeks, or even years. Similarly, it remains difficult to estimate which concentrations of organic pollutants should be used when running *in vitro* experiments because some of these molecules accumulate at very high concentrations in specific organs or tissues. Finally, the studies that have been performed so far on the IFN-I response do not take into account a potential "cocktail" effect, which corresponds to synergistic interactions between organic pollutants at very low doses. Based on epidemiological and toxicological data, it would be particularly relevant to test combinations of pollutants on different populations of primary immune cells and measure the IFN-I response, particularly in pDCs that are specialized in the production of IFN- I. Finally, all of these studies should be extended to type III interferons (IFN-III or IFN-λ) that were more recently discovered. Indeed, these cytokines are induced and signal by mechanisms very close to IFN-I, and also participate in the antiviral response.

In the future, an essential step will be to confront *in vitro* studies with epidemiological data, both in humans and animals, to establish potential correlation between *in vivo* levels of organic pollutants, the IFN-I response, and the incidence of viral diseases. It should also be determined if correlations exist between contamination levels and the

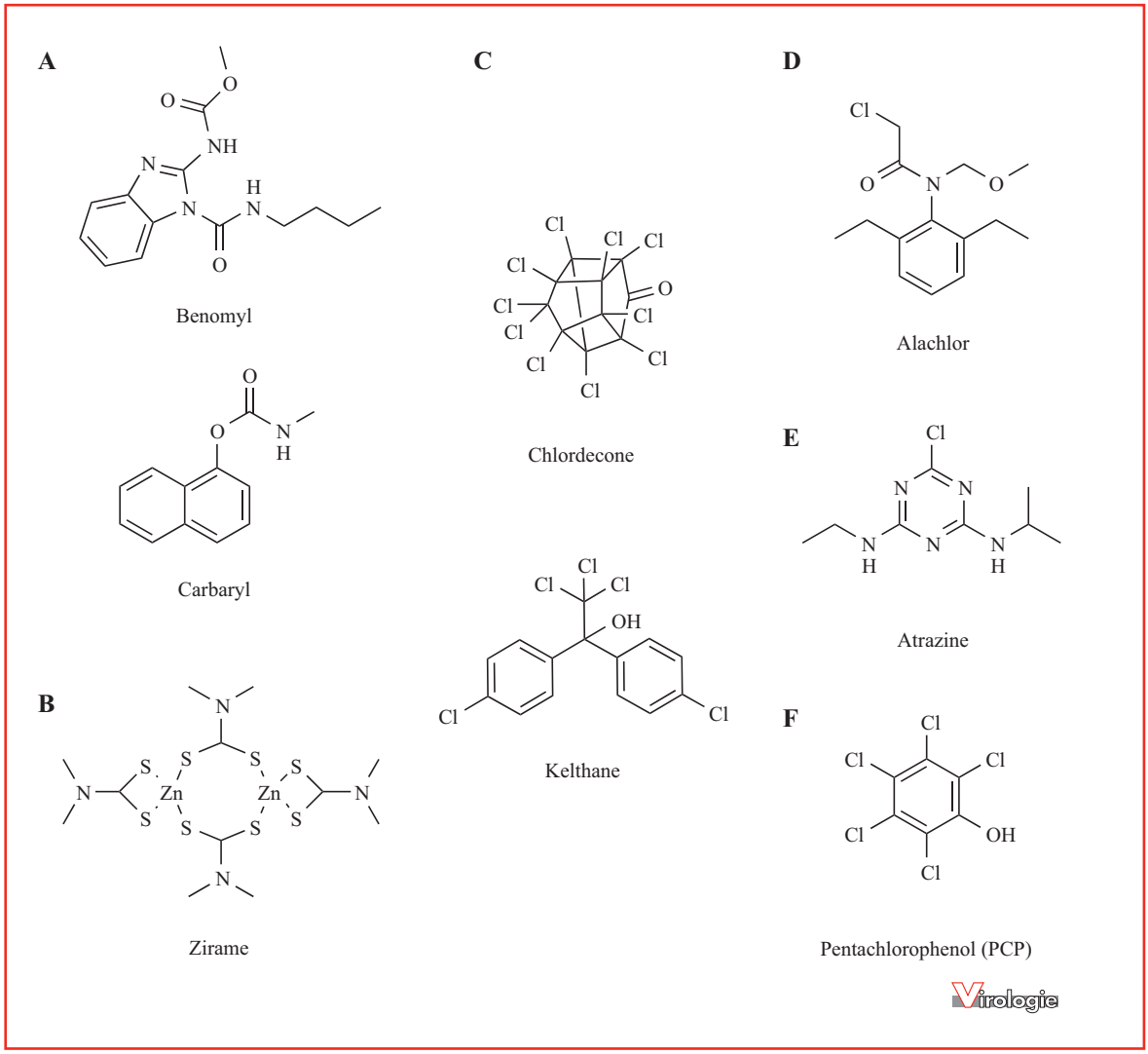

Figure 4. Structures of different pesticides that affect the innate antiviral response. (A) Structures of benomyl (fungicide) and carbaryl (insecticide) of the carbamate family. (B) Structure of ziram, a fungicide of the dithiocarbamate family. (C) Structure of two organochlorine insecticides, chlordecone and Kelthane. (D) Structure of alachlor, an herbicide of the chloroacetamide family. (E) Structure of atrazine, an herbicide of the triazine family. (F) Structure of pentachlorophenol that can be used as a fungicide in different materials.

development of autoimmune or auto inflammatory diseases involving IFN-I. It will also be essential to better understand the mode of action of these compounds on the innate antiviral response, which for example, will require to determine the binding mode of organic pollutants to AhR (for which we only have homology-based models; *figure 5*) and to estrogen receptors, or their capacity to induce some oxidative stress or DNA damages. Finally, if several organic pollutants apparently increase the morbidity and mortality associated with certain viral infections, the mechanisms involved are probably complex: alterations of innate and adaptive immune responses, modification of metabolism at cellular and system levels, effects on the microbiota, etc. In a polluted environment that weakens individuals and makes them more susceptible to viral infections, is the inhibition of the IFN-I response a key factor? Would this inhibition of the innate antiviral response promote the emergence of new viruses? By combining epidemiological studies and the analysis of the immunomodulatory effects of pollutants using high-throughput *in vitro* assays, it will be possible to address these questions. Ultimately, these studies could lead to some improved regulation and labeling of chemicals, taking into account their effects on the immune response.

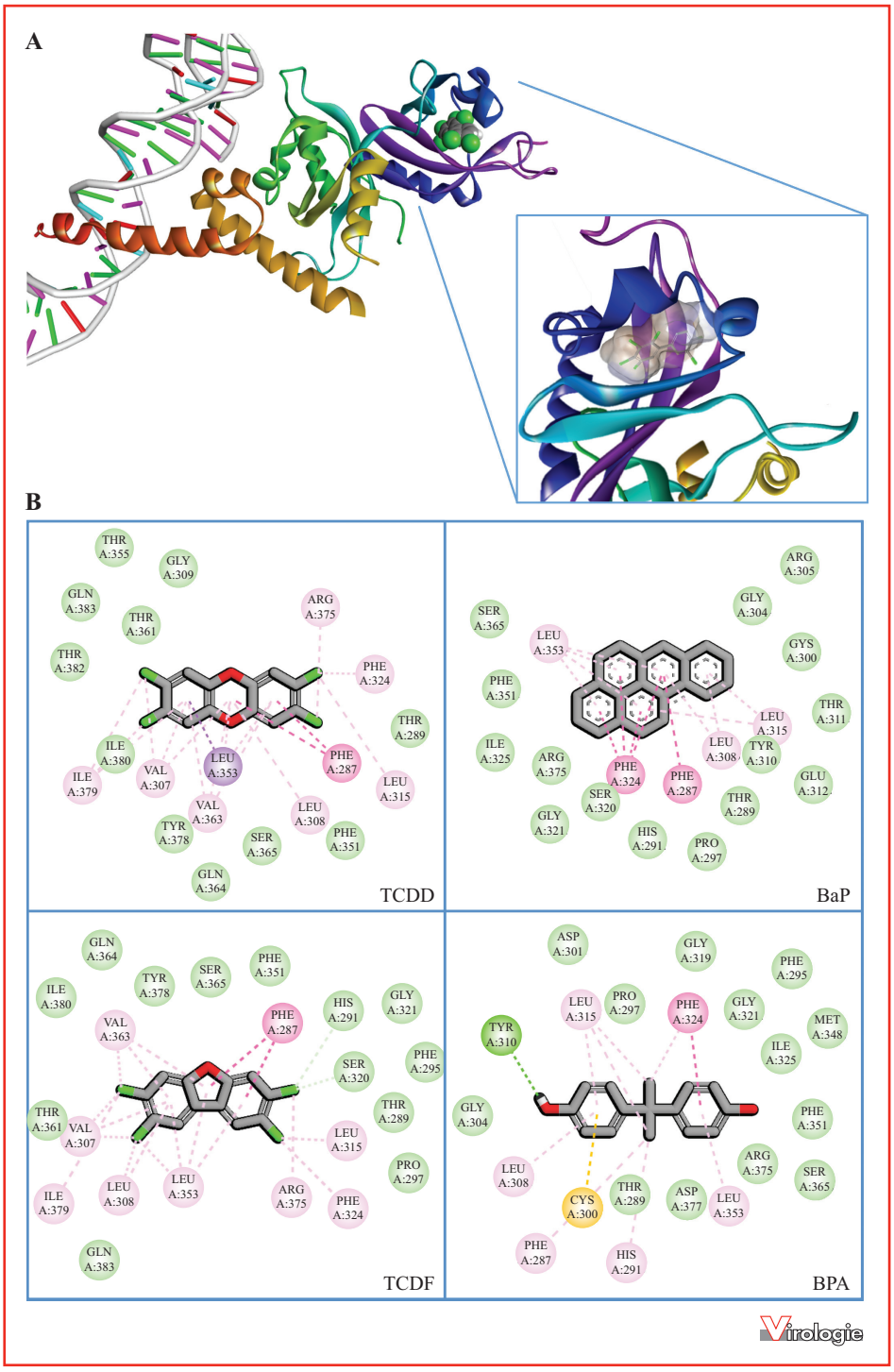

Figure 5. Molecular docking of ligands on a model by homology of AhR. (A) The 3D representation of the AhR protein was constructed by homology from a known partial structure of AhR (pdb code 5NJ8) and NPAS3 protein (pdb 5SY7). The compounds were docked on the interaction zone defined by Goryo *et al.* [59] using cDocker (rigid protein/flexible ligand). (B) The residues involved in the interaction have been identified and are illustrated in 2D interaction diagrams for TCDD, BaP, TCDF, and BPA. They are colored according to the type of interaction performed with the ligand: green for Van der Waals; pale pink, pink and violet for hydrophobic alkyl, π-π or π-σ; yellow for sulfur-π.

This work was supported by the Agence Nationale pour la Recherche (Programme ChemInnate), Campus France (Programme CEDRE) and the Lebanese National Council for Scientific Research.

Conflicts of interest : none.

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