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Review

Interactions between polycyclic aromatic hydrocarbons in complex mixtures and implications for cancer risk assessment

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ABSTRACT

In this review we discuss the effects of exposure to complex PAH mixtures *in vitro* and *in vivo* on mechanisms related to carcinogenesis. Of particular concern regarding exposure to complex PAH mixtures is how interactions between different constituents can affect the carcinogenic response and how these might be included in risk assessment. Overall the findings suggest that the responses resulting from exposure to complex PAH mixtures is varied and complicated. More- and less-than additive effects on bioactivation and DNA damage formation have been observed depending on the various mixtures studied, and equally dependent on the different test systems that are used. Furthermore, the findings show that the commonly used biological end-point of DNA damage formation is insufficient for studying mixture effects. At present the assessment of the risk of exposure to complex PAH mixtures involves comparison to individual compounds using either a surrogate or a component-based potency approach. We discuss how future risk assessment strategies for complex PAH mixtures should be based around whole mixture assessment in order to account for interaction effects. Inherent to this is the need to incorporate different experimental approaches using robust and sensitive biological endpoints. Furthermore, the emphasis on future research should be placed on studying real life mixtures that better represent the complex PAH mixtures that humans are exposed to.

Contents

Abbreviations: AhR, aryl hydrocarbon receptor; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; B[*a*]P, benzo[*a*]pyrene; B[*a*]P_{eq}, B[*a*]P-equivalent; Chk1, checkpoint kinase 1; CT, coal tar; CYP, cytochrome P450; DE, diol epoxide; DNA-PK, DNA-dependent protein kinase; DPE, diesel particulate extract; DPM, diesel particulate matter; EFSA, European Food Safety Agency; H2AX, H2A histone family member X; HEL, human embryonic lung; IARC, International Agency for Research on Cancer; MAF, mixture assessment factor; NTP, National Toxicology Program; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; PCN, polychlorinated naphthalene; PEF, potency equivalency factor; ROS, reactive oxygen species; RPF, relative potency factor; SRM, standard reference material; TBA, tumor bearing animal; TEF, toxic equivalency factor; UD, urban dust.

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1. Introduction

The field of mixture toxicology and the experimental analysis of chemical mixtures have undergone a significant expansion borne out of the need to understand the effects of exposure on human health. Historically, experimental approaches have focused on understanding the effects of individual or simple combinations of chemicals. However, this approach is being superseded in favor of investigating the effects of complex mixtures and understanding of the interactions between different chemicals. A review published in the early 1990s summarized the then status of toxicological assessment of complex mixture effects (Mauderly, 1993). That the issues pertaining to complex mixture assessment presented at that time are still as prevalent today highlights the challenges faced by researchers in this field.

Humans are exposed to complex mixtures of chemicals through various sources including occupational settings, the environment, cigarette smoking, vehicular exhaust emissions, pharmaceuticals, lifestyle products and foodstuffs. Exposure occurs over a prolonged period of time and generally at low levels. Typically this exposure includes a vast assortment of chemicals including, but not limited to, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and naphthalenes (PCNs), pesticides and heavy metals. There is however a significant lack of information available today regarding complex mixture toxicology.

A major group of chemicals that are found in complex mixtures are the PAHs, a family of more than 1500 compounds (NTP, 2012) comprised of two or more fused aromatic rings, found both in their native and substituted forms (*i.e.* methylated, oxygenated or nitrated) (Fig. 1). PAHs are ubiquitous environmental pollutants that are formed as a result of incomplete pyrolytic processes and to which humans are exposed through inhalation, ingestion and dermal absorption. Links between human exposure to complex PAH mixtures and development of diseases including cancer, and respiratory and cardiovascular diseases have been described previously (ATSDR, 1995; Boffetta et al., 1997; IARC, 2010; Peters et al., 2001; Pope et al., 2002). Despite their structural similarities, PAHs vary greatly in their carcinogenic potency, with both individual and complex mixtures of PAHs classified as possible or probable human carcinogens by the International Agency for Research on Cancer (IARC) (IARC, 2010).

Assessment of the carcinogenic risk to humans of exposure to PAHs often involves comparison to benzo[*a*]pyrene (B[*a*]P) and two approaches are commonly used (Boström et al., 2002; Pufulete et al., 2004). In the first approach B[*a*]P is used as a surrogate marker to determine quantitative risk estimates for mixtures of PAHs in air (WHO, 2000) or food (EFSA, 2008). The second approach is component-based in which the potency of different PAHs is expressed relative to that of B[*a*]P (assigned a nominal value of 1). The assigned values for the different PAHs are termed relative potency factors (RPF) or toxic equivalency factors (TEF)/potency equivalent factors (PEF). Detailed description of the differences between how the factors are derived have been published previously (Backhaus et al., 2010; U.S.EPA, 2000). An important issue regarding the component-based approach is that due to a lack of data not all PAHs have been assigned potency values and this is likely to lead to misestimating of the risk. Principles pertaining to how risk assessments could be applied to complex PAH mixtures have been proposed (ATSDR, 2004; EC, 2009; Flowers et al., 2002; IPCS, 2009; NTP, 2012; SCHER et al., 2012; U.S.EPA, 2007, 2010), and indeed a recent research proposal testing the health effects of complex PAH mixtures has been approved (NTP, 2012). However, it is accepted that due a number of limiting factors (discussed in detail below) this is likely to lead to misestimating of the risk to human health.

The aim of this paper is to review what is currently known about the effects of interactions between PAHs in complex mixtures on mechanisms related to carcinogenesis. We then discuss ways and advantages of studying complex PAH mixtures and how such findings could be applied to future risk assessment.

2. Carcinogenic effects of PAHs

The binding of PAHs to DNA and the associated effects that occur as a result is considered the major mechanism of PAH-induced mutagenesis and carcinogenesis. Like many chemical carcinogens, PAHs require activation through a series of enzymatically-catalyzed reactions to form their active metabolites (Conney, 1982; Huberman et al., 1976; Sims et al., 1974). The CYP family of enzymes, in particular CYP1A1, 1A2 and 1B1, are primarily involved in bioactivation of PAHs to their reactive intermediates (Pelkonen and Nebert, 1982; Shimada and Fujii-Kuriyama, 2004; Shimada et al., 1996). Many PAHs are ligands for the aryl hydrocarbon receptor (AhR), which has different roles involved in metabolism including regulation of the different bioactivating and detoxifying enzymes (Baird et al., 2005; Nebert et al., 2004). For example, B[*a*]P is activated through a three-step enzymatic mechanism involving initial metabolism by CYP enzymes to B[*a*]P-7,8-epoxides, followed by conversion to B[*a*]P-7,8-diols by epoxide hydrolase, and final transformation to the ultimate reactive B[*a*]P-7,8-diol-9,10-epoxide metabolites, again by CYP enzymes (Conney, 1982). This mechanism of activation has also been shown for other PAHs. An additional metabolic pathway is the aldo-keto reductase mechanism that activates PAHs to redox-active o-quinone derivatives which might also have tumorigenic and mutagenic activities (Park et al., 2008; Penning et al., 1999; Zhang et al., 2012). Although many substituted PAHs are also targets for CYPs, their bioactivation often involves additional steps. For example, nitrated PAHs (such as 1-nitropyrene) require initial nitroreduction to primary amines before they are metabolized by CYPs to hydroxylamines and activated by conjugation reactions (Chou and Fu, 1983; Djuric et al., 1986).

The tumorigenic and mutagenic activities of many PAHs have been linked to the ability of their diol epoxide (DE) metabolites to bind covalently to exocyclic amino groups on purine bases to form either stable bulky PAH-DE-DNA adducts or depurinating adducts which are released from DNA leaving abasic sites (Rogan et al., 1993; Sims and Grover, 1974; Szeliga and Dipple, 1998). Quinones can also bind DNA and form stable N2-dG or N2-dA adducts (Penning et al., 1999; Shou et al., 1993) or depurinating N7-dG adducts (McCoull et al., 1999). PAHs exhibit a large variety in their ability to form adducts with DNA which may be a result of different structural conformations. Most carcinogenic PAHs contain either a bay or a fjord region within their structure (Fig. 1). PAHs containing a bay region (*i.e.* B[*a*]P) are rigid