Published in final edited form as:
*Environ Res*. 2021 March ; 194: 110662. doi:10.1016/j.envres.2020.110662.

# The role of epidemiology studies in human health risk assessment of polychlorinated biphenyls

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## Abstract

Polychlorinated biphenyls (PCBs) are a public health concern given evidence that they persist and accumulate in the environment and can cause toxic effects in animals and humans. However, evaluating adverse effects of PCBs in epidemiologic studies is complicated by the characteristics of PCB exposure. PCBs exist as mixtures in the environment; the mixture changes over time due to degradation, and given physicochemical differences between specific PCB congeners, the mixture that an individual is exposed to (via food, air, or other sources) is likely different from that which can be measured in biological tissues. This is particularly problematic when evaluating toxicity of shorter-lived congeners that may not be measurable by the time biological samples are collected. We review these and other issues that arise when evaluating epidemiologic studies of PCBs and discuss how epidemiology data can still be used to inform both hazard identification and dose-response evaluation.

### Keywords

Polychlorinated biphenyls; Neurodevelopment; Exposure; Risk assessment; Human health

## 1. Introduction

Polychlorinated biphenyls (PCBs) are organic halogenated compounds which were manufactured and marketed worldwide, including in the United States under the trade name Aroclor between about 1930 and 1977. Due to their electrical insulating properties, chemical stability, and relative inflammability, PCBs were used in myriad products and applications, including electrical equipment (e.g., capacitors, transformers), sealants and caulking compounds, and as coolants and lubricants (ATSDR 2000). However, due to evidence that they persist and accumulate in the environment and can cause toxic effects, the US Environmental Protection Agency (EPA) banned the manufacture of PCBs, and phased out most PCB uses in 1979 under the Toxic Substances Control Act (TSCA) (40 CFR 761) (http://www2.epa.gov/aboutepa/epa-bans-pcb-manufacture-phases-out-uses). In addition,

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PCBs were included as part of the original 'dirty dozen' in the Stockholm Convention, with the goal of eliminating usage of existing PCB-containing equipment by 2025 http://chm.pops.int/Implementation/IndustrialPOPs/PCBs/Overview/tabid/273/Default.aspx.

Despite the phaseout of most PCB products, PCBs remain an important health concern due to the potential for ongoing exposure from contaminated environments; humans continue to be exposed to PCBs throughout life, including early life exposures through breastfeeding (van den Berg et al., 2017), through diet (Schecter et al., 2010), and inhalation (Lehmann et al., 2015). In addition, PCBs can also be *inadvertently* generated during industrial processes, such as pigment production (Rodenburg et al., 2015). PCB exposure has been associated with both cancer and noncancer health effects, based upon both animal toxicology studies and human epidemiology studies (ATSDR 2000; IARC 2015). The toxicity of PCBs has been reviewed by both national and international health agencies, considering both cancer (IARC 2015) and non-cancer health effects (ATSDR 2000, 2011); these reviews describe numerous health endpoints sensitive to PCB exposure.

The evaluation of the human health effects resulting from PCB exposure is challenging. The nature of PCB exposure is complex due to the large number of congeners (n = 209), changes in mixture profiles in different environmental media and body burden over time, multiple routes of exposure and challenges in quantifying shorter-lived PCB congeners. Further, health effects may vary widely across mixtures, since the physicochemical properties of PCB congeners vary with the number and position of chlorine atoms. PCBs may exert estrogenic, antiestrogenic, and other properties depending upon the structure of the specific congeners involved. Consequently, there have been suggested groupings of PCBs that correspond to their structure and activity (e.g. (Wolff et al., 1997)) in order to better investigate associations with health outcomes. For example, animal toxicology studies demonstrate that PCB mixtures with larger percentages of both higher-chlorinated and dioxin-like congeners are the most potent for inducing hepatocellular neoplasms, hepatic histopathological changes, and thyroid follicular-cell hyperplasia in rodents (Mayes et al., 1998); however, such mixtures have similar potency as lower-chlorinated PCB mixtures in immunotoxicity and neurotoxicity assays (Harper et al., 1995; Seegal et al., 1991).

Despite the hundreds of epidemiological studies performed to evaluate health effects in PCB-exposed populations, there are challenges in using these data for risk assessments. However, the epidemiology database can offer valuable contributions at all stages of human health risk assessment, from identification of human health hazards and quantification of the exposure-response relationship, to estimates of exposure and resulting characterization of risks to specific populations. Characteristics specific to the human health risk assessment of PCBs will be outlined below, along with opportunities and challenges related to using epidemiological data for dose-response evaluation of PCB mixtures and health outcomes in exposed populations.

## 2. Challenges in PCB exposure assessment for epidemiology studies

As noted above, the 209 different PCB congeners may vary widely in their persistence and toxic effects. Adding to the complication, there are differences between the PCB congeners

and proportions present in technical (produced) mixtures, compared with the mixtures currently in the environment and in humans. In the environment, PCB mixtures undergo weathering processes such as adsorption to sediments/soils and volatilization (reviewed in (Erickson 2018)). PCBs can also undergo degradation (by microorganisms or photolysis), which can impact the number and location of chlorine atoms in different PCB molecules. These changes over time can impact bioavailability and bioaccumulation, which can result in dramatic differences in the congener profiles of PCB mixtures found in various exposure sources (e.g., human milk, contaminated fish, indoor air) and the mixtures found in assessments of body burden (e.g., blood samples). As noted by Erickson, "*Weathering tends to skew patterns or, especially with biodegradation, selectively deplete/enrich certain congeners*", further cautioning that quantitation of weathering over time is unlikely to be useful given the number of uncertainties associated with the process (Erickson 2018).

Once taken up into the body (through ingestion, inhalation, or dermal absorption), PCB mixtures may be further altered due to biological processes. The measurable levels of PCBs in a biological sample reflect not just the original external exposure, but also time elapsed since exposure occurred, ability to metabolize PCBs (determined in part by genetics), past and current body composition (e.g., changing amount of body fat), and for females, pregnancy and lactation history and current pregnancy status (Mitro et al., 2015; Nickerson 2006; Rogan et al., 1986). PCB congener half-lives in humans vary widely (from a few hours to over a decade) (Ritter et al., 2011); thus, as time passes the measured body burden *due to the original exposure* will be dominated by longer-lived PCBs even if the original exposure contained shorter-lived congeners as well. It is not always clear if measured body burden reflects continuous low-level exposure to longer-lived compounds with bioaccumulation, or recent exposure to shorter lived compounds, or some combination of the above. PCBs can accumulate in lipid tissue but vary in lipophilicity, which generally increases with increasing chlorination. PCBs are transported largely via lipids in blood to all tissue compartments throughout the body (Matthews and Dedrick 1984), and the specific tissue distribution is expected to vary depending upon the congener mixture, the initial exposure dose, the route of exposure, and characteristics of the exposed individual.

Another complication for risk assessment integration and application is that there is considerable variation in how exposure to PCBs is measured across epidemiology studies. Some studies define 'exposed' populations based upon knowledge of a specific exposure event (for example, work in a plant producing Aroclor mixtures or birth to a mother consuming contaminated rice oil in the Yusho or Yu-Cheng populations). Others may combine measures of PCBs in environmental media – for example, concentrations in fish tissue or in air – with information on frequency, duration and intensity of exposure, to characterize the exposure experience of study participants with regards to PCB exposure. For dietary exposure, participants may complete food frequency questionnaires to quantify the amount of contaminated fish consumed during critical exposure windows. Any type of exposure estimate can be subject to measurement error and subsequent exposure misclassification when continuous exposures are categorized into groups. In the examples above for fish tissue and air exposures, there is an assumption that the PCB mixture in the environmental medium (media) evaluated is predictive of the internal mixture that induces any associated health effects. One advantage of external exposure sampling data (especially

if source- and/or media-specific) is that it can directly inform prevention or regulation. For example, if levels of a PCB mixture in an environmental medium are found to cause harm, removing or reducing those levels or access to the medium should remove or reduce the associated health risks. However, such studies are not common in the literature and may only measure a limited number or type of PCB congeners, which may not adequately represent the PCB mixture responsible for observed health effects.

More commonly, studies characterize PCB exposure using measurements in biological samples such as blood, breast milk or adipose tissue. The specific analytic method used varies across studies, as does the collection, preparation and storage of biological samples. The number of congeners measured varies widely and may depend on the analytic method used. For example, older studies more commonly measured PCBs based upon Aroclor standards, and many studies only measure a small number of congeners. With improved analytic methods, newer studies may be able to consider a larger number of congeners with similar sample volumes. In studies utilizing biomarkers (as with studies using external measures of exposure), there is also an assumption that the measured PCB mixture represents the mixture that is responsible for any noted health effects. However, there is also a recognition that the measured mixture is perhaps (or likely) less representative of the original PCB mixture present in environmental media due largely to varying rates of metabolism and elimination of PCB congeners in humans, as noted above. As described in a review (Grimm et al., 2015), certain PCB congeners (typically lower chlorinated ones) may be undetectable or detected at very low levels due to rapid metabolism; however, this does not mean that the original exposure dose was small, or that these specific congeners do not contribute significantly to the original mixture or to toxicity; indeed, the authors state that "*Low blood levels are often misinterpreted as an indication for low exposure and therefore low relevance when in fact they should be taken as an indication of continuous, steady-state, and therefore potentially significant overall exposure to compounds that have a high propensity to be bioactivated to potentially harmful metabolites*." (Grimm et al., 2015).

Few studies have attempted to investigate the difference between PCB mixtures measured in biospecimens compared with environmental media, but one example comes from the Airborne Exposure to Semivolatile Organic Pollutants (AESOP) Study which measured a large number of congeners in children's blood as well as in indoor and outdoor air samples (Ampleman et al., 2015; Koh et al., 2015). This sampling strategy allowed authors to investigate differences by indoor versus outdoor air, rural versus urban settings, and demographic characteristics. Importantly, the investigators noted that blood sampling data demonstrated exposure to more-volatile lower molecular weight congeners not frequently reported in epidemiological studies.

Given the lipophilic nature of PCBs, most epidemiology studies utilize PCB measurements that are lipid adjusted, or otherwise consider lipid content in evaluating associations with health outcomes. A 2009 study by Gaskins and Schisterman (2009) outlined various approaches for lipid consideration and potential for resulting bias under various sets of causal assumptions and concluded that no one approach was uniformly preferred. Rather, the biospecimen type and statistical modeling approach used should be considered when choosing the appropriate adjustment. Other workshop-based efforts have recommended that

epidemiological studies report both whole volume and lipid-adjusted concentrations (LaKind et al., 2014). It may be particularly important to consider the method of lipid adjustment when evaluating exposure in utero and via breastmilk, given the mobilization of adipose depots that accompany pregnancy and lactation (releasing previously stored PCBs into circulation) and high lipid content of human breastmilk.

Some investigators have addressed the changing exposure milieu during pregnancy and lactation using multiple measures based on serially collected biospecimens or multiple types of biospecimens. This is especially critical to match corresponding sample collection with known or anticipated critical exposure periods for specific outcomes, such as prenatal exposure evaluation for certain neurodevelopmental outcomes. Other studies have estimated exposure levels based on models of varying complexity. For example, postnatal exposure via breastfeeding may simply consider PCB concentrations measured in a single breastmilk sample multiplied by estimated milk ingestion for a given time period. Other studies may use more elaborate physiologically based pharmacokinetic (PBPK) models to estimate exposure. PBPK models developed for PCBs estimate concentrations in various tissue compartments under different exposure scenarios considering the main drivers of PCB toxicokinetics: lipophilicity, binding to liver proteins (e.g., cytochromes, aryl hydrocarbon receptor [AhR]), and rate of elimination (due to metabolism or fecal excretion).

## 3. Potential for human epidemiology studies to support PCB dose-response assessment

Even with the challenges of evaluating exposure to PCBs, epidemiology studies are still capable of providing valuable insights. Considering exposure measurement error as a general limitation, it is important to remember that even without individual-level quantitative exposure measurements (i.e., only categorical group-level data are available that separate those ‘highly exposed to PCBs’ from ‘low exposure’ or ‘no exposure’), valid hazard identification of health effects can be made. Further, it may be possible to apply externally derived exposure data (e. g., biomarkers from a subset of the population) both to verify exposure trends observed on the group level, and even to develop individual-level exposure estimates in qualitative or semiquantitative categories (Claus Henn et al., 2010; Ritz and Costello 2006).

When exposure measurement error is suspected, information is often available in epidemiology studies that can help characterize the direction or magnitude of errors to estimate their impact on the association between exposure and health outcome and aid causal inference. For example, one well-known approach to measurement error adjustment within epidemiological studies is regression calibration (Fraser and Stram 2001). Finally, even if the exact magnitude of exposure is not well estimated, as long as the exposure measure(s) can be used to accurately rank or characterize individuals’ exposure experience, this level of information can support valid hazard identification and, to some degree, considerations of exposure-response.

Many epidemiology studies measure a limited number of PCB congeners, and older studies using packed column analytic methods may have no congener-specific information

available. A 2017 study measured all 209 congeners in a small number of blood and plasma samples (Kraft et al., 2017) in order to determine whether the use of a smaller subset of congeners, with application of a correction factor, could adequately represent 'total' PCB exposure. Of note, the authors describe only one other human study that measured all 209 congeners (Marek et al., 2014), and state that "*Comparable test results with such a large number of measured PCB congeners in blood or plasma have rarely been published to date*." The authors concluded that measuring six indicator PCBs would allow investigators to reasonably estimate total PCB body burden but noted that their results may not be generalizable; further, this approach does not allow the identification of toxicity due to specific congeners.

Risk assessments are often based on specific environmental sources or routes of exposure. Thus, extrapolation or back-calculations may be needed to make epidemiological study results amenable to dose-response analyses used for risk assessment. Specific to PCBs, a major concern outlined above is the mismatch between the original mixture, the mixture present in environmental media during the exposure period of interest, and the mixture measured in biological samples. Importantly, different magnitude and direction of error in the measurement of each specific congener can complicate evaluation of underlying exposure measures in risk assessment applications and they may lead to bias of reported effect estimates either towards or away from the null (Zeka and Schwartz 2004). In response to this concern, some studies have used reverse dosimetry to extrapolate from measures of PCBs in serum or breast milk to measures of external exposure (Ulaszewska et al., 2012; Verner et al., 2008). In these papers, the researchers utilized measures of specific persistent, stable (e.g., those with long half-lives) congeners – this is a strength in the sense that the estimated external dose for these congeners may be more accurate compared with shorter-lived compounds, and also the congeners used are very commonly measured in epidemiology studies. So, for any health effects associated with exposure to those specific congeners, it may be possible to establish associations with health outcomes and even estimate dose-response relationships. The main drawback is that the three most commonly measured congeners do not represent a complete environmental PCB mixture, which would typically consist of many different congeners of varying physicochemical and toxic properties, and other unmeasured congeners may contribute significantly to toxicity. Thus, if any associations with health effects are found, it's likely that other unmeasured PCBs with similar structure and activity are also contributing to that effect, and that consequently the effect measure could be either under or over estimated. However, this method does provide valid insight into health effects associated with these commonly measured and highly prevalent congeners.

For epidemiology studies of early life effects, exposure occurs through the uterine environment and breastmilk. Thus, fetal or infant exposure can be captured by characterizing PCB mixtures present in the uterine environment (placental tissue, amniotic fluid, meconium, cord blood) and PCBs in breastmilk. This allows an opportunity to examine the relationships between different PCB profiles (specific congeners present and concentrations) in both mothers and infants. A number of studies have taken this approach, and results from these investigations can not only inform the interpretation of epidemiological associations and address the relevance of timing and frequency of sampling versus known critical

exposure windows, but they may also be able to provide valuable data to inform the development of more sophisticated pharmacokinetic models to describe the transfer of PCBs from mother to infant through pregnancy and lactation. However, more research in this area is needed. Existing studies are not consistent in determining whether higher or lower chlorinated congeners more readily cross the placenta, and others have suggested that PCB transfer is affected by parity (reviewed in (Mitro et al., 2015). Higher chlorinated congeners are larger and have a higher molecular weight, hence would be expected to diffuse more slowly through tissues, but that difference in rate of diffusion may not be significant relative to the time-course of in utero exposure. For example, in subsequent pregnancies, a woman's body mass, in particular fat fraction, is likely to be greater than during the first pregnancy (Lassek and Gaulin 2006). This would affect overall distribution between her body and the fetus; however, a mechanism that would affect the distribution between maternal and fetal blood, the ratio of PCB concentration across the placenta, has not been proposed. Careful biomonitoring, including multiple samples from the same subjects to reduce the impact of inter-individual variability, under conditions in which exposure is reasonably stable, will likely be needed to determine with certainty the impact of the degree of congener chlorination and other factors, including parity, on the distribution of PCBs between mother and placenta.

In addition, there is still the difficulty of determining the maternal exposures that led to the observed PCB mixtures experienced by the fetus or infant. As noted above, if the mother's PCB exposures occurred mainly in the past (not ongoing) then the mixture in her body would likely be dominated by longer-lived, higher chlorinated PCB congeners, and this is the more 'stable' mixture that would be measured in her breastmilk, for example. In the case of ongoing exposure, more labile congeners could be a significant 'component of the mixture; to have a better chance of capturing the changing exposure milieu, it might be necessary to take multiple measurements throughout pregnancy and lactation at time windows important for the developmental outcome of interest. Nevertheless, studying early life effects may offer a good opportunity to more completely characterize PCB exposures because of the shorter timeframe for critical exposure windows, especially since many diseases may have fetal origins (Barker 2007).

## 4. Evaluating PCB dose-response relationships using epidemiology studies

As noted above, there are hundreds of studies evaluating health effects due to PCB exposures (https://hero.epa.gov/hero/index.cfm/project/page/project_id/384). We focus here on selecting studies from the epidemiology database that can inform not only hazard identification, but also dose-response analysis. General considerations for evaluating epidemiology studies have been outlined in Cooper et al., (2016) and the Systematic Review Protocol for Polychlorinated Biphenyls Noncancer IRIS Assessment (available at: https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=237359), and cover domains such as study design, participant selection, quality of exposure and outcome evaluation, appropriate analytic approach, and sensitivity of the study to detect an association and/or discern if an exposure-response relationship exists. Specific to the use of epidemiology studies used for

dose-response evaluation of PCB-related health effects, a key characteristic to consider is the relevance of the exposure paradigm. The exposure experienced by study participants must be characterized with adequate precision to characterize exposure rank or magnitude. Another consideration for the relevance of the exposure paradigm is that studies evaluating exposures at environmental levels are preferred over studies evaluating only high levels of exposure. For this reason, epidemiologic studies conducted among general population groups are preferred; less preferred are those conducted in occupational settings or among populations with only high exposures due to (for example) industrial accidents, which would result in more uncertainty in dose-response modeling and requires additional extrapolation. Another consideration is the comparability of the exposed and referent group. As in all epidemiology studies, the goal is to identify groups which are comparable with respect to the distribution of confounders, but that differ in exposure status. Specific to PCBs, this raises the issue of confounding or effect measure modification by co-pollutants; in realistic environmental contaminant exposure scenarios, many persistent and bioaccumulative chemicals such as PCDDs, PCDFs, chlorinated pesticides, MeHg, and PCBs share some physical properties that contribute to their collective persistence and bioaccumulation. Therefore, populations exposed to PCBs are often co-exposed to non-PCB contaminants via the same environmental matrices. The effect of these co-exposures, with regard to presence, direction and magnitude, may depend on the outcome observed. For example, PCDDs, PCDFs, and non-ortho PCBs share the ability to activate the AhR and induce “dioxin-like” toxicological effects. Thus, co-exposure to these chemicals is likely to increase the dioxin-like effects observed and hence make it very difficult to distinguish the effects of PCBs. Conversely, some important sources of PCB exposure (i.e., fish and breast milk) offer nutritional benefits that could mask adverse effects (e.g. neurotoxicity) of PCBs. Epidemiology studies that either measure and account for co-exposures in their analysis, or that are conducted among groups unlikely to have such co-exposures, would provide stronger evidence for health effects of PCB mixtures.

Given these considerations, we now present an example of selecting epidemiology studies for dose-response analysis, focusing on neurodevelopment in early life. This outcome domain was selected for the example because of the large number of available studies, many of which come from longitudinal cohorts evaluating multiple PCB congeners (and relevant co-pollutants) in relation to outcomes assessed using valid and reliable approaches.

### 4.1. Considerations when identifying studies suitable for dose-response evaluation

The database of studies evaluating PCB exposure in relation to neurodevelopment in infancy and early life was identified using a literature search and screening strategy as outlined in the Systematic Review Protocol for Polychlorinated Biphenyls Noncancer IRIS Assessment (available at: https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=237359). This database was reviewed to identify a few studies that would be considered adequate for use in hazard identification based on the guidelines and considerations referenced in preceding sections, and that could potentially inform dose-response evaluation of PCB exposure and decrements in neurodevelopment. The four publications selected for this case study (Table 1) are all based on prospective (birth) cohorts, from Spain (Forns et al., 2012a), (Forns et al., 2012b), Greece (Kyriklaki et al., 2016), and the United States (New York) (Stewart et al.,

2008). These studies specifically reported on cognition evaluated using ‘gold standard’ instruments for evaluation of infants (the Bayley Scales of Infant Development, BSID) or children (McCarthy Scales of Children’s Abilities, MSCA; Wechsler Intelligence Scale for Children-third revision, WISC-III). Each study evaluated multiple PCB congeners in pre or perinatal tissue samples (maternal blood, cord blood, placenta) and considered co-pollutants and key potential confounders in the analysis.

### 4.2. Measures of exposure

The four studies discussed here were different with regard to the exposure metrics used. Two (Forns et al., 2012a; Kyriklaki et al., 2016) used PCBs measured in maternal blood samples taken during pregnancy. One used cord and child (at 4 years of age) blood (Forns et al., 2012b), and one used placental tissue (Stewart et al., 2008). These all, with the exception of child blood levels at 4 years of age, reflect exposure in utero and early infancy (which would be an etiologically relevant time period for prenatal effect on neurodevelopment). Three studies used a subset of more persistent congeners for exposure-response modeling (all included 138, 153 and 180); the INMA cohort studies noted that PCBs 28, 52 and 101 were measured, but not considered further due to the fact that nearly all were below the LOD. A strength of the Oswego study (Stewart et al., 2008) is that a larger number of congeners was measured in placental tissue (investigators measured 75 peaks corresponding to 136 congeners). Three of the four studies reported exposure levels (not regression model results) using wet-weight concentrations; for the fourth study (Forns et al., 2012a), information from an ancillary publication (Lopez-Espinosa et al., 2015) was used to estimate the unadjusted median PCBs in maternal serum, in order to more readily compare across studies.

Although the analytic methods and exact congeners measured varied by study, when considering four of the common PCB congeners (118, 138, 153, 180), in all cases PCB 153 had the highest average concentration, followed by 138 and 180, with 118 having the lowest average concentration. As discussed above, it may be that this pattern is due to the longer half-life of PCB 153, and not necessarily because cohorts were exposed to more PCB 153 in the environment. This limitation was common across the cohorts. In the Oswego cohort, there were many statistically significant associations between higher PCB levels and decrements in IQ, even with the longest time elapsed (9 years) between exposure and outcome evaluation. If child neurodevelopment is more stable after 3 years of age, outcomes evaluated in later years may be more useful for dose-response evaluation. In the Rhea cohort, the effect measures were generally negative, but were significant only for working memory, perhaps due to the lower levels of PCBs and other cohort-specific factors. Findings in the INMA cohort included decrements in PDI score (younger ages), and some decrements with individual PCB congeners measured in cord blood and lower scores on the MSCA outcomes evaluated at 4 years of age. However, levels of PCBs in child blood (that is, evaluated at the time of outcome evaluation) did not show associations with MSCA. PCB levels measured in maternal blood during pregnancy (as was done for one of the INMA studies and the Rhea study) are most proximal to in utero exposure while cord blood and placenta measures may capture the period closer to birth; these types of measures may therefore be more suitable for evaluating dose-response relationships with neurodevelopmental effects in early childhood. More distant measures of exposure such as

blood samples from childhood, may be less useful given the opportunity for changing PCB mixture levels and composition over time when the critical window of exposure is thought to be in utero.

## 5. Discussion and future research needs

This example highlights considerations and challenges of heterogenous exposure measures and illustrates the potential for human epidemiology studies to inform human health risk assessment, specifically the dose-response stage to quantify the magnitude of effect associated with increased exposure. A simple example using four epidemiology studies demonstrated decrements in neurodevelopmental outcomes evaluated up to 9 years of age, with increased early life exposure. Given that each study utilized a prospective design and accounted for potential confounders using an appropriate statistical analysis, the main factors differentiating them were the outcome evaluation instrument and age, and the method of PCB exposure assessment. Each study had different strengths and limitations in this regard – for example, the Oswego study evaluated the greatest number of PCB congeners, but also the greatest time elapsed between exposure and outcome evaluation (Stewart et al., 2008). Two studies evaluated maternal blood PCB levels during pregnancy, but for only a limited number of PCB congeners (Forns et al., 2012a; Kyriklaki et al., 2016). Overall, dose-response information from any of these studies is useful for hazard assessment, but looking at them in combination provides a basis for stronger conclusions about the relationship between PCB exposure in early life and neurodevelopmental effects.

The existing database of epidemiology studies demonstrates a multitude of possible health hazards associated with PCB exposures, but in many cases the magnitude of risk is uncertain due to variation in the specific mixture(s) to which the study population was exposed, and how PCB exposure was measured. Studies have used both external (e.g., environmental media) and internal measures of PCB exposure, as well as exposure inferred from occupational, residential, or other ancillary information. Even among studies that use a biomonitoring approach, the specific tissue(s) sampled, frequency and timing of exposure relative to critical exposure windows, and analytic approaches, can make it challenging to compare results across studies. Even if current body burden is perfectly measured, there may be differences in subsequent health risk due to other exposure-related factors – for example, Longnecker et al., (2003) note that high rates of exposure for shorter time periods may be more toxic than chronic low level exposure, despite resulting in equivalent PCB levels in biological samples at time of measurement. Further, as described at length above, current body burdens may not reflect cumulative exposures because of biological processes and transformation over time, which is particularly problematic if studies measure only a small number of congeners, or neglect to measure more labile, lower chlorinated congeners with shorter half-lives (Marek et al., 2014; Ritter et al., 2011).

Consequently, future research needs to center around the evaluation and dissemination of PCB exposure. A recent paper by Burns et al. outlines considerations for epidemiology studies that aid in translating findings to risk assessment (Burns et al., 2019); the discussion on exposure characterization is particularly relevant for PCBs, and includes such recommendations as describing source to intake pathways, more completely describing

exposure characteristics for the study population, and describing potential existence and magnitude of exposure measurement error. Other concrete suggestions include having researchers present findings using both wet-weight and lipid-adjusted measurements and provide descriptive statistics for all congeners measured when possible (not just the summed or 'total' PCB measure). For longitudinal (cohort) studies of PCBs, taking measurements at multiple time points (e.g., study entry, during follow-up, end of follow-up) would also provide valuable information on stability of measured body burden over time and consequences for estimated associations with health outcomes. In some situations, it may be possible to use stored biological samples from biospecimen repositories (e.g., the Women's Health Initiative or the UK Biobank) to measure PCB body burden in the past and relate to subsequently measured health outcomes. Aside from time considerations, in order to more fully characterize mixtures, it is necessary to measure multiple congeners of varying range and activity. One way to utilize data from existing studies would be to reprocess stored biological samples to measure a larger number of congeners, or perhaps new samples from the same environment or individuals can be collected for such analyses. This could be particularly useful for characterizing shorter-term (gestational and lactational) exposure. Longer-term exposure scenarios would still suffer from an inability to measure less-persistent congeners that were eliminated before the samples were collected. Thus, researchers need to consider exposure assessment methods that are suitable not only for 'typical' persistent pollutants, but additionally methods that are suited for shorter-lived (typical 'non-persistent') compounds. Another suggestion for studies in the planning stages (or newly launched studies), would be to collect a variety of samples for PCB analysis, including environmental media (e.g., air sampling for inhaled PCBs) as well as biological samples, as has been done in the AESOP study, for example (Ampleman et al., 2015). This could lead to a better understanding of the similarity or differences between PCB mixtures in the exposure source, and what is measured internally. As noted above, measurements in media associated with health impacts could also be more readily translated into actionable 'clean up' or risk reduction actions. Finally, further development of tools and evaluation of data using these tools to assess similarity of mixtures, to extrapolate across routes of exposure, and to characterize the toxicokinetics of PCBs once in the body, will aid understanding of the measures that are most relevant for evaluating associations with health outcomes, as well as for risk characterization and risk management. The example shown here illustrates how these tools may enhance the utility of existing epidemiology studies for human health risk assessment of PCBs and also offers recommendations for future improvements.

## Acknowledgements

We thank Michael Wright, Thomas Bateson, and Ruth Etzel for help improving the manuscript. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*Environ Res*. Author manuscript; available in PMC 2022 March 01.

**Table 1**

Characteristics of studies selected for dose-response evaluation of PCB exposure and neurodevelopment in infancy and early life.

| Study Citation | Forns et al. (2012a) | Forns et al. (2012b) | Kyriklaki et al. (2016) | Stewart et al. (2008) |
|---|---|---|---|---|
| Study Population | Prospective (birth) cohort. The INMA (INfancia y Medio Ambiente [Environment and Childhood]) Project (1997–2008) enrolled women in seven areas across Spain. Inclusion: aged ≥16 years, intention to deliver at regional hospital, singleton pregnancy, ability to communicate in Spanish or regional languages, no assisted conception. | | Prospective birth cohort. The Rhea Study (2007–2008) was designed as a population-based sample of pregnant women from the prefecture of Heraklion, Crete, Greece. Inclusion: Good understanding of Greek language, aged ≥16 years, singleton live birth. | Prospective (birth) cohort. The Oswego Newborn and Infant Development Project (New York, USA, 1991–1994) enrolled pregnant women at 20 week sonogram visit at county's only OB practice. Inclusion: aged ≥18 years, or 17 years with parental consent. |
| Sample Size | Includes INMA cohorts from: Valencia, Sabadell, and Gipuzkoa Initial enrollment: n = 2150<br>Assessed at ~14 months: n = 1801<br>Included in this analysis: n = 1391 | Includes INMA cohort from: Menorca Initial enrollment: n = 482<br>Assessed at 4 years: n = 418<br>Included in this analysis: n = 355 (cord blood), n = 278 (child serum at 4 years) | Initial enrollment: n = 1363<br>Assessed at 4 years and included in this analysis: n = 689 | Initial enrollment: 602<br>Assessed at 9 years and included in this analysis: n = 156 |
| Age | 14 months | 4 years | 4 years | 9 years |
| PCB congeners measured | 28, 52, 101, 118, 138, 153, 180 Note that 28, 52 and 101 had >95% of values < LOD, while PCB 118 had detect rate of 23%; these were not considered further, so sum of PCBs includes (138, 153, 180) | 28, 52, 101, 118, 138, 153, 180 Note that 28, 52 and 101 had all values < LOD and not considered further, so sum of PCBs includes (118, 138, 153, 180). | 118, 138, 153, 156, 170, 180 | 136 total; focus on 118, 138, 153, 180 |
| Method of PCB measurement | Serum samples analyzed using gas chromatography with electron capture detector. Gas chromatography coupled to mass spectrometry used for confirmation. Relative standard deviation < 15% for all PCBs. Limits of detection ranged from 0.01 to 0.71 ng/mL. | (Details from Ribas-Fito et al. (2003)). Serum samples analyzed using gas chromatography with electron capture detector. Gas chromatography coupled to mass spectrometry used for confirmation. Between-assay coefficient of variation ranged from 6 to 11% for all PCBs. Limit of detection was ~0.10 ng/mL. | Serum samples analyzed using gas chromatography coupled to quadrupole mass spectrometry.<br>Limit of quantification ranged from 6 to 10 pg/mL. | Placental samples analyzed using gas chromatography with electron capture detector and dual-column confirmation. All congener values were reported by the laboratory and were not censored below detection limits |
| Tissue | Maternal blood samples (between the 7th and 26th weeks of pregnancy) | Cord blood; Child blood at 4 years | Maternal blood samples (3rd-4th month) | Placental tissue |
| Median (IQR) for summed PCBs | 102.7 (66.2, 145.3) ng/g lipid Estimated median wet weight (Lopez-Espinosa et al., 2015)[a]: 0.60 ng/mL | Cord: 0.71 (0.54, 0.97) ng/mL<br>4-year: 0.80 (0.56, 1.21) ng/mL | 0.32 (0.22, 0.48) ng/mL | 1.5 (1, 2.1) ng/g placental tissue 221.0 (147.7, 303.4) ng/g lipid |
| Median (IQR) for individual congeners | (ng/g lipid)<br>118: 6.4 (5.6, 7.8)<br>138: 26.1 (16.3, 38.4)<br>153: 43.4 (28.6, 61.5)<br>180: 31.1 (19.9, 45.8)<br>Estimated median wet weight (ng/mL) (Lopez-Espinosa et al., 2015)[b]<br>118: 0.04<br>138: 0.15<br>153: 0.25<br>180: 0.18 | (Cord, ng/mL)<br>118: 0.06 (0.05, 0.1)<br>138: 0.14 (0.10, 0.20)<br>153: 0.18 (0.14, 0.26)<br>180: 0.13 (0.10, 0.19) | (pg/mL)<br>118: 17.8 (12.0, 25.3)<br>138: 68.8 (45.3, 102.0)<br>153: 125.7 (85.5, 188.3)<br>156: 6.5 (3.0, 10.7)<br>180: 67.0 (44.5, 104.9)<br>170: 33.8 (21.9, 53.5) (ng/mL)<br>118: 0.018 (0.012, 0.025)<br>138: 0.069 (0.045, 0.102)<br>153: 0.126 (0.086, 0.188)<br>156: 0.007 (0.003, 0.011)<br>180: 0.067 (0.045, 0.105)<br>170: 0.034 (0.022, 0.054) | (ng/g tissue)<br>118: 0.09<br>138 + 163+164: 0.12<br>153: 0.13<br>180: 0.06 *(other medians in supplemental material of original article)* |

| Study Citation | Forns et al. (2012a) | Forns et al. (2012b) | Kyriklaki et al. (2016) | Stewart et al. (2008) |
|---|---|---|---|---|
| Outcome Instrument (measures evaluated) | Bayley Scales of Infant Development (BSID: Mental Development Index [MDI], Psychomotor Development Index [PDI]) | McCarthy Scales of Children's Abilities (MSCA: General Cognitive Index, Verbal, Perceptive-Performance, Memory, Quantitative, Motor, plus 7 additional scales [Executive Function, Working Memory, Memory Span, Verbal Memory, Functions of Posterior Cortex, Fine Motor, Gross Motor]) | McCarthy Scales of Children's Abilities (MSCA: General Cognitive Index, Verbal, Perceptive-Performance, Memory, Quantitative, Motor, plus 4 additional scales [Executive Function, Working Memory, Memory Span, Functions of Posterior Cortex]) | Wechsler Intelligence Scale for Children (WISC-III: Full Scale IQ, Verbal, Performance, Freedom from Distractibility, Verbal Comprehension, Perceptual Organization) |
| Outcome Measurement Details | Administered at health care center (with mother present) by one of eight trained, blinded psychologists. State interrater reliability for examiners was 0.9–0.91. Scores were adjusted for age and normalized to yield mean (SD) of 100 (15) points. | MSCA version adapted to Spanish population but with some new measures added (Executive functions, Working memory, Visual and verbal span, Verbal memory, Gross and Fine motor skills, Cognitive functions of posterior cortex). MSCA administered by one of two trained neuropsychologists, state that protocol implemented to minimize interrater variability but without details. Mean (SD) of general cognitive index was 100.4 (15.2). | Administered during 4-year clinical visit in hospital setting. State that 'translation and cross-cultural adaptation of the MSCA was performed according to internationally recommended methodology, and that scores were age-standardized. Children evaluated by one of two randomly assigned psychologists with reported high inter-observer reliability. Evaluators noted the quality of the MSCA administration. | Administered at SUNY-Oswego lab by one of four blinded examiners (all trained by single school psychologist. Interrater reliability estimated for certain subtests and ranged from 0.90 to 1.00. Median (range) for FSIQ was 101 (62, 135). |
| Evaluation of confounding | Covariates retained in the model if associated with BSID score (p-value < 0.05) or if inclusion resulted in a ≥10% change in the effect estimate.<br>Age accounted for with use of age-adjusted scores.<br>Included in final model of MDI: cohort, sex, main caregiver, maternal country of birth, maternal social class, birth height, gestational age, breastfeeding duration.<br>Included in final model of PDI: cohort, paternal social class, gestational age. | Covariates retained in the model if associated with MSCA GCI (p-value < 0.05) or if inclusion resulted in a ≥10% change in the effect estimate.<br>Age accounted with use of age-adjusted scores.<br>Included in final model: examiner, child age and sex, maternal social class, folic acid supplementation during pregnancy, maternal cigarettes during pregnancy, paternal education, duration of breastfeeding | Certain covariates included as a priori potential confounders, also included those which changed the effect estimate by ≥10%.<br>Age accounted for with use of age-adjusted scores.<br>Included in final model: maternal triglycerides and cholesterol, sex, quality of assessment, examiner, maternal age, maternal education, smoking during pregnancy, parity. | Method 1: include covariates associated with outcome (p < 0.20) and/or which change beta coefficient by≥10%. Method 2: include covariates that were associated with both exposure and outcome (p < 0.20), but not in the causal pathway.<br>Age accounted for with use of age-adjusted scores.<br>Included in final model: maternal test outcomes (IQ, Wisconsin Card Sort, CPT, NES2); parental education; SES; birth order; parental age, weight, and height; the HOME environment; maternal stress and illnesses; birth weight, head circumference, Ballard score (physical); maternal cigarette smoking and second-hand smoke exposure; DDE levels; maternal tea and caffeine use; child daycare; maternal depression; marital status.<br>**Note – child sex was evaluated, but was not associated with the outcome measures.* |
| Co-pollutants considered | Multipollutant models for each outcome also adjusted for DDE and HCB. | Multipollutant models for each outcome also adjusted for DDE and HCB. | HCB and DDE measured in maternal serum. | Total Hg (maternal serum); MeHg, DDE, Mirex, HCB (placenta); lead (placenta, postnatal) |
| Analysis | Multiple linear regression model with log10-transformed PCBs. Present adjusted beta coefficients with 95% CIs for 10-fold increase in exposure. State that missing covariate data were multiply imputed and results from imputed dataset were not meaningfully different from complete case | Multiple linear regression models with log-transformed PCB concentrations. Present adjusted beta coefficients and SEs per log-unit of exposure. Evaluated potential effect modification by duration of breastfeeding and child fish intake. | GAMs used to evaluate shape of exposure-response relationship; indicated possible nonlinearity so categorized PCB exposure as high (<90th percentile) or reference (all others) for multiple linear regression models. Present adjusted beta | Multiple linear regression models with continuous PCBs, but also used categorical exposure to evaluate shape of dose-response relationship. Evaluated significance of cubic and quadratic terms, and nonmonotonicity using Sidak reversal tests. Present distribution of |

| Study Citation | Forns et al. (2012a) | Forns et al. (2012b) | Kyriklaki et al. (2016) | Stewart et al. (2008) |
|---|---|---|---|---|
| | analysis (data shown in Supplement of original article). Evaluated potential effect modification by breastfeeding and sex using product terms. | | coefficients and 95% CIs. Also evaluated potential effect modification by sex, maternal prepregnancy BMI and maternal TSH during pregnancy, using product terms. Sensitivity analyses to exclude preterm birth. | exposure and outcome. Present p-values from linear trend test using ANCOVA (F test). Present standardized and adjusted beta coefficients and p-values. |
| Adjusted beta coefficient with 95% CI or SE, for summed PCBs (statistically significant results are in bold text) | MDI: 0.11 (−1.39, 1.17)<br>MDI Multipollutant: 0.44 (−1.92, 1.05)<br>PDI: **1.24** (−**2.41**, −**0.07**).<br>PDI Multipollutant: 1.22 (−2.63, 0.09). | *Cord blood: Significant associations for individual congeners, but not for summed PCB measure*<br>GCI: 2.44 (1.53)<br>Verbal: 1.77 (1.53)<br>Quantitative: 2.14 (1.57)<br>Perceptual-Performance: 2.43 (1.53)<br>Memory: 2.52 (1.55)<br>Motor: 1.53 (1.52)<br>*Child blood: No significant associations for summed PCBs or individual congeners.* | GCI: 2.36 (−6.52, 1.79)<br>Verbal: 1.41 (−5.57, 2.75)<br>Quantitative: 3.44 (−7.83, 0.95)<br>Perceptual-Performance: −2.37 (−6.62, 1.88)<br>Memory: 0.43 (−4.69, 3.83)<br>Motor: 0.22 (−4.44, 4.89)<br>Executive Function: 3.58 (−7.85, 0.69)<br>Working memory: **4.62** (−**9.10**, −**0.14**)<br>Memory span: 0.34 (−1.10, 4.78)<br>Posterior cortex functions: 1.09 (−5.22, 3.04) | *(Standardized/nonstandardized)*<br>FSIQ: **0.17/−2.90**<br>Verbal IQ: **0.21/−4.10**<br>Performance: 0.04/(not reported)<br>Verbal Comprehension: **0.18/−3.30**<br>Freedom from distractibility: **0.24/−4.40**<br>Perceptual Organization: 0.04/(not reported) |

[a] Lopez-Espinosa et al. report that "*We used enzymatic techniques to determine total cholesterol and triglycerides, and total serum lipid concentrations were calculated as described by Phillips et al. Means ± SDs of total lipid contents in maternal (n = 473) and cord (n = 486) serum were 5.8* ±2.3 *and 2.6 ±0.5 mg/mL, respectively*." The median wet weight was estimated as (lipid-adjusted value * [August 5, 1000]) = (102.7 * [August 5, 1000]) = 0.60 ng/mL.

[b] Lopez-Espinosa et al. report that "*We used enzymatic techniques to determine total cholesterol and triglycerides, and total serum lipid concentrations were calculated as described by Phillips et al. Means ± SDs of total lipid contents in maternal (n = 473) and cord (n = 486) serum were 5.8 ± 2.3 and 2.6 ±0.5 mg/mL, respectively*." The median wet weight for each congener was estimated as (lipid-adjusted value * [August 5, 1000]).