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Reproductive and developmental effects of PCBs in fish: a synthesis of laboratory and field studies

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Abstract. Polychlorinated biphenyls disrupt reproduction and development in fish in the laboratory. However, the effects of PCBs on fish exposed in the field are often unclear. In this review 'effective concentration' ranges for reproductive and developmental toxicity of PCBs are developed by synthesizing published data. There are many limitations to this data synthesis approach, which are addressed in detail, including the estimation of tissue concentrations of PCBs and interspecies differences in response to PCBs. The effective concentration ranges are 25–70 ppm in the liver of adult fish, and 5–125 ppm in the bodies of larvae for Aroclor 1254 (A1254) and 0.3–5 ppm in adult fish and 1.3 ppm in eggs for PCB 77. The effective concentrations for A1254 are within range of PCB concentrations associated with similar adverse effects found in field studies. In addition, they fall within the range of A1254 concentrations measured or estimated for fish from highly contaminated sites within the United States. Effective concentrations of PCB 77 are also within the range of congener 77 concentrations estimated for some of the most contaminated fish within the United States.

1. Introduction

The toxicology of polychlorinated biphenyls (PCB) is a topic of great interest for wildlife and fisheries researchers and managers. Since the early 1970s dozens of field and laboratory studies have focused on the effects of either Aroclor mixtures or PCB congeners in fish, and an even greater number of studies report PCB concentrations in fish from lakes, rivers, bays and oceans around the world. The basic question compelling many of these studies is 'do PCBs have the potential to cause reproductive and developmental toxicity in fish exposed in the field?' There is clear evidence of PCB toxicity in fish from laboratory studies. The majority of these studies focus on the reproductive and developmental toxicity of PCBs. However, despite the fairly large number of studies on PCBs in fish, the question of the potential for PCBs to affect fish in the field remains elusive in many cases.

I attempt to address this question by reviewing, evaluating and synthesizing the currently available research literature and by developing estimates of fish tissue PCB concentrations above which reproductive and/or developmental effects are likely in fish exposed to PCBs in the field (i.e. determine 'effective concentrations'). There are three main components to this review 1) literature review 2) data

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synthesis and development of 'effective PCB concentrations' and 3) comparison of PCB concentrations in fish from selected field sites to the effective concentrations. Since the effects of PCBs on reproductive and developmental processes in fish are the primary focus of this review, I first provide a brief overview of PCB toxicology followed by some of the basic reproductive processes in fish known to be affected by PCBs. A detailed review of data and literature focusing on the effects either caused by PCB mixtures or PCB congeners in fish is then presented using several summary tables. Using these data I develop estimated 'effective concentration' ranges for reproductive and developmental toxicity in fish caused by the PCB mixture Aroclor (A1254) and PCB congener 77 (PCB 77). Since routes of exposure, dose regimen, and study duration can be highly variable among studies, where possible I relate reproductive and developmental endpoints to PCB concentration in a single common tissue. Liver tissue in adults was selected for this purpose because of the importance of the liver in reproductive processes and its capacity to accumulate PCBs.

The effective concentrations for PCB toxicity in fish are then compared to PCB concentrations associated with reproductive and developmental toxicity in several field studies, which are summarized in table format and to PCB concentrations either measured in fish liver (or measured in fillets and estimated in liver tissue) from contaminated field sites. The methods used to estimate liver PCB concentrations in laboratory and field studies are discussed in detail later in this review. There are several sites around the country that are highly contaminated with PCBs. Of these sites, there was sufficient published data to compare the effective concentration range for A1254 to fish from the Hudson River (NY), Lake Hartwell (SC/GA) and New Bedford Harbor (MA). Recent reports indicate that fish from locations along the Housatonic River (MA) are among the most highly contaminated in the country, although there was insufficient information to estimate concentrations of A1254 from the PCB totals reported in this study [20].

PCB congener data for individual fish was available for only two of the sites listed above, the Hudson River and New Bedford Harbor. Thus, effective concentrations of PCB 77 are compared to concentrations of PCB 77 measured in fish from the Hudson River and New Bedford Harbor. Finally, in order to provide a more direct comparison of exposure and toxicity of dioxin-like PCB congeners, toxic equivalency quotients (TEQ) are calculated and discussed for Hudson River fish (TEQs for fish from New Bedford Harbor were calculated in previous studies by Elskus et al., [31] and Black et al., [9]). Synthesizing and comparing data on A1254 and congener 77 required an analysis of data from many different laboratories, which for various reasons used different species of fish for their studies. The last section of this review is devoted to a comparison of the wide variety of species used for laboratory and field studies, and species which are potentially at risk in a contaminated system. Again, this section focuses on fishes of the Hudson River.

As discussed earlier, many different types of information from a variety of studies are synthesized to develop effective concentration values for PCBs in fish. There

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are clearly limitations when synthesizing data from so many different studies, many of which not only use different species but different exposure regimens and focus on different endpoints. The accuracy of the effective concentrations is limited by the degree to which PCB concentrations in tissues for laboratory studies that did not report concentrations could be estimated; interspecies differences in response to PCBs, since the studies reviewed used a variety of fish species; differences in response to PCBs resulting from timing of exposure in relation to the maturation cycle, as the studies reviewed varied exposure timing; and differences in the route of exposure (i.e., water, injection, or diet) used in each laboratory study.

In addition there are other limitations to consider when using these effective concentrations to assess potential harm in field animals. Common problems associated with relating laboratory studies to field situations include: differences in the species and routes of exposure; differences in length of exposure (laboratory exposures are usually shorter than the typically lifetime exposure of a field animal); uncertainties associated with comparing toxic effects following exposure of naive laboratory animals with field animals that may be exposed throughout their lifetime; comparing exposures to single chemicals with the complex mixtures of contaminants often encountered in the field; and comparing results from exposure to commercial Aroclor mixtures to exposure from a potentially different mixture of PCB congeners (as a result of differential metabolism or bioaccumulation) encountered in the field. These limitations are acknowledged and addressed where appropriate throughout this review. Many of the topics addressed in these discussions clearly warrant further research.

1.1. PCB structure and toxicity

PCBs are mixtures of chlorinated biphenyl congeners. There are three categories of PCBs: PCBs that are chlorinated in two or more ortho positions, those that are chlorinated in only one ortho position (mono-ortho), and PCBs lacking any ortho chlorination, known as non-ortho or co-planar PCBs (Fig. 1). Early work on PCBs focused on PCB mixtures such as A1254, 1260 or 1248. Later work focused on individual PCB congeners, with the greatest emphasis on the co-planar PCBs. Most recently the focus is shifting back to PCB mixtures and to congeners other than the co-planars because, although the co-planar PCBs may be the most potent congeners, other congeners may have important toxic effects as well [89].

PCBs cause a wide range of toxic effects across species from mammals to fish. There is a large amount of information on toxicity of PCBs and several reviews on this topic are available [26,27,44,101]. The toxicity of PCBs varies depending on the PCB mixture and the species, sex and age of the exposed animal. In mammals, PCB exposure causes wasting syndrome, reproductive failure, teratogenicity, infertility in males, immunotoxicity (including thymic and splenic atrophy), hypothyroidism, chloracne, neurobehavioral effects, liver damage and induction of cytochrome P450; additionally PCBs may act as promoters of liver cancer [43–

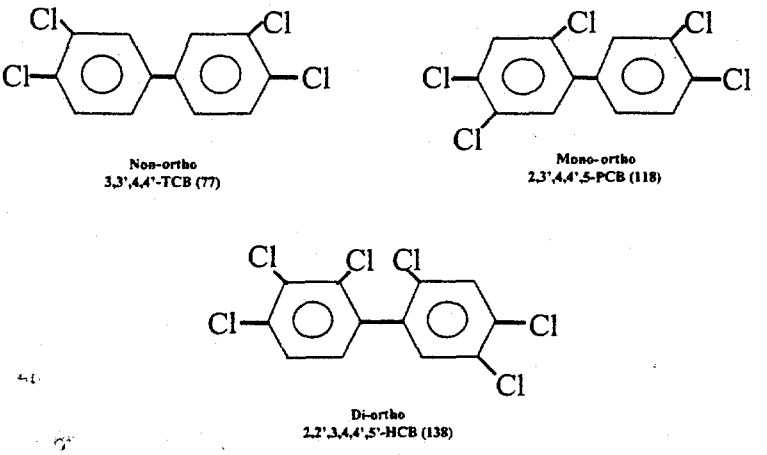

Fig. 1. The toxicity of PCBs is related to their structure. Those that are not chlorinated in either ortho position (e.g. PCB 77), referred to as non-ortho or co-planar PCBs are among the most toxic in fish.

45,89,101]. Many of these effects also occur in fish including reproductive and developmental toxicity, immunotoxicity, liver damage, neurobehavioral effects, effects on thyroid hormones and induction of cytochrome P450 [26,27,33,34,74, 82,135]. Recent studies have indicated that PCBs may alter retinoid concentrations as well [93,99].

1.2. Toxicity of co-planar PCBs

The toxicity of PCBs follows a structure activity relationship [101,106], with the greatest potency associated with the co-planar PCBs [106,135]. The toxicity of the co-planar PCBs is believed to be mediated via the arylhydrocarbon receptor (AhR), similar to dioxin. The AhR is a highly evolutionarily conserved receptor found in most vertebrate species [48,49]. This means that it is fairly similar across species from fish to mammals, and that it may respond to specific ligands (e.g., some PCBs, polyaromatic hydrocarbons (PAH), dioxin) in a similar manner across species. Dioxin is the most potent known ligand for the AhR, and it is currently accepted by most toxicologists that the toxicity of dioxin is primarily mediated by the AhR. It is also accepted by most toxicologists that the toxicity of other compounds, including some PCB congeners, is mediated via the AhR.

The fact that the AhR receptor is so highly conserved evolutionarily explains many of the shared effects of AhR-active chemicals across species, although there can be large differences in interspecies sensitivity to these compounds [28, 101,102]. In addition, there can be differences in the structure activity relationship among species as demonstrated by the greater potency (i.e., ability to activate AhR) of the mono-ortho chlorinated PCBs (e.g., PCB 105) in mammals compared to teleosts [129,134]. In mammals, activation of the AhR results in induction of cyto-

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chrome P450 (one of the most well understood responses), and many other effects including wasting syndrome, liver damage, immunotoxicity, teratogenesis, and reproductive disorders including reduced sperm counts, delayed puberty in females, and urogenital malformations (reviewed in [21,43]). Many of these effects including wasting syndrome, reproductive disorders, teratogenesis and immunotoxicity also occur in fish.

Since the effects of AhR-active (or co-planar) PCBs are considered to pose the greatest threat to wildlife including fish, there has been a great emphasis on techniques that provide an assessment of the risk from exposure to these congeners. Because the toxicity of individual PCB congeners can vary dramatically it is very difficult to assess the potential health risk to an organism based on tissue concentrations of congener mixtures. Thus, the toxic equivalency factor approach (TEF) was developed to facilitate risk assessment and management. This approach provides a measure of the toxicity of all AhR-active chemicals based on the same 'unit' – the toxicity relative to dioxin. The TEF is the potency of a chemical relative to dioxin, and allows for conversion of tissue concentration data (of AhR-active chemicals) to equivalent units of dioxin (TEQs: toxic equivalents). Many different *in vivo* and *in vitro* techniques have been used to develop TEFs. Most recently the European Center of Environmental Health of the World Health Organization and the International Program on Chemical Safty (ECEH-WHO and IPCS) determined 'consensus TEFs' [129]. These consensus TEFs are used to calculate TEQs for fish from the Hudson River. The process used by the ECEH-WHO and IPCS to develop consensus TEFs, is discussed in greater detail later in this review. The TEQs of several different AhR-active congeners can then be added together to provide a TEQ for the whole mixture, as shown in the equation below:

$$TEQ = \Sigma([PCB_i] \times TEF_i)$$ [1]

Finally, cytochrome P4501A (CYP1A) is an important and sensitive marker of exposure to AhR-active contaminants. One of the long-standing questions in this field is the relationship between induction of CYP1A and health effects. Induction of CYP1A is AhR-dependent. Inducers (e.g., dioxin, PAHs, some PCBs) bind with the AhR, then the receptor-ligand complex interacts with DNA resulting in the production of AhR gene products, and one of these gene products leads to the production of CYP1A. There are several reviews on CYP1A induction by various environmental contaminants, including PCBs [14,117,118]. Currently, any direct relationship between CYP1A induction or inhibition and health effects remains unclear.

1. This approach can also be used to calculate *TEQ*s for mixtures containing other AhR-active compounds including *PCDD*s, and *PCDF*s:
$TEQ = (\Sigma[PCB_i] \times TEF_i) + (\Sigma[PCDD_i] \times TEF_i) + (\Sigma[PCDF_i] \times TEF_i)$.

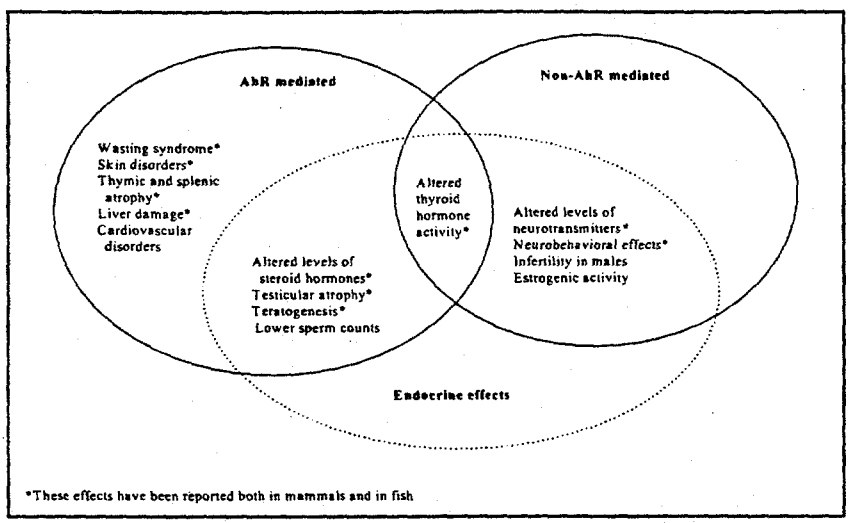

Fig. 2. Toxicity of PCBs grouped by putative mechanism. PCBs that act by binding with the Ah receptor (AhR) cause effects typical of AhR binding toxicants [21,43–45]. In addition there are several important effects of PCBs that are non-AhR mediated [89]. The majority of both AhR and non AhR effects result in disruption of the endocrine system.

1.3. Non-AhR mediated toxicity

There is a growing body of research showing that there are important toxic effects not mediated via the AhR. These effects include neurotoxicity (and altered levels of neurohormones), some thyroid hormone effects, and estrogenic activity in mammals [89]. Some of these effects are common to fish, including altered levels of both thyroid hormone and neurotransmitters [67,68,74,82]. Figure 2 provides a summary of the toxic effects of PCBs, grouped by the putative mechanism of action as described above. The effects caused by PCBs that are mediated by interaction with the AhR are listed under the AhR mediated mechanism. The toxic effects that are caused by PCBs not known to interact with the AhR are listed under the non-AhR mediated mechanism. In some cases, the same endpoint (e.g., altered thyroid hormone activity) may occur by different mechanisms (i.e., AhR and non-AhR mediated). Many of the AhR and the non-AhR effects can be categorized as endocrine effects. The endocrine system is an important regulatory and integrating system which relies on hormone production, transport and action to control functions such as growth, development, and reproduction (reviewed in [128]). Thus, PCBs are endocrine disruptors that act on several different levels of the endocrine system.

The concern for effects of PCBs in fish (and other wildlife species) is a result of exposure to these compounds in the field. I develop effective concentrations for PCB toxicity by synthesizing data from many different laboratory studies. When evaluating laboratory studies, there is always the question of relevance to PCB ex-

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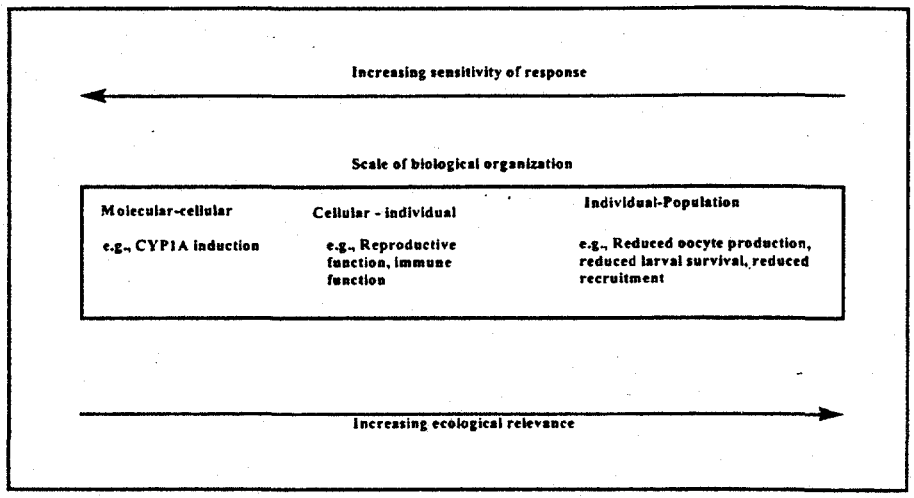

Fig. 3. Responses to environmental contaminants by levels of biological sensitivity versus ecological relevance. When considering biological endpoints for toxicity and monitoring studies, there is often a tradeoff between sensitivity to toxicants and ecological relevance. Evaluation of reproductive effects can provide an endpoint that is both sensitive and relevant.

posure in the field. One of the most common critiques of many toxicity studies is the use of high (pharmacological) doses rather than lower 'environmentally relevant' concentrations. However, as shown in the results sections of this review, this is not the case with many PCB studies. For sites that are highly contaminated with PCBs (e.g., New Bedford Harbor, Hudson River, Housatonic River, Lake Hartwell), and even some lesser contaminated sites, the PCB concentrations used in the majority of laboratory studies are within the range of PCB concentrations measured in wild-caught fish.

1.4. Focus on Reproductive and developmental effects

The focus on reproductive and developmental toxicity is the result of two considerations 1) these endpoints are sensitive to PCBs and other anthropogenic contaminants and 2) they are ecologically relevant endpoints. Reproductive effects are defined as any change in the hypothalamic-pituitary-gonadal-liver axis and include alterations in sex steroid hormones, gonadotropins, gonad growth, and vitellogenin production. Developmental effects refer primarily to embryo and larval growth and survival. Most studies do not continue beyond the larval stage and therefore do not evaluate potential behavioral effects or long-term effects of PCBs on survival of exposed offspring.

One of the primary concerns for both wildlife and fisheries toxicologists is the effects of chemical contaminants in both individual animals and on whole populations. When monitoring the effects of chemical contaminants, there are an array of

endpoints ranging from cellular level effects that are very sensitive to chemical exposure to changes in population size or structure that are generally less sensitive to chemical exposure (Fig. 3). There is often a trade-off between early detection of exposure and ecological relevance when assessing the effects of contaminants in the field. Cytochrome P450 induction, for example, is a very sensitive indicator of contaminant exposure, but its relationship to health effects is unclear. A change in population size or structure, however, is clearly relevant, although populations often are severely impacted by the time these changes can be observed.

One approach to the tradeoff between early detection of exposure and ecological relevance is to select endpoints that are fairly sensitive, but that can be linked to health effects on individuals, or to population size or recruitment. In general, reproductive and developmental endpoints satisfy these criteria. Although the relationship between some endpoints such as altered steroid hormone concentrations and individual health or population size is currently unclear, other changes such as reduced gonad size (particularly in female fish), egg production, and embryo and larval survival could impact population size.

1.5. Reproduction and maturation in fish

Maturation in fish is under neuroendocrine control [91,123]. This process is illustrated in Fig. 4. Basically, in maturation the brain produces gonadotropin releasing hormone (GnRH) in response to environmental and endogenous signals. The pituitary then responds to GnRH by producing gonadotropins (GtH), which then act on the gonads (ovary or testes). Gonadotropin secretion is also modulated by other

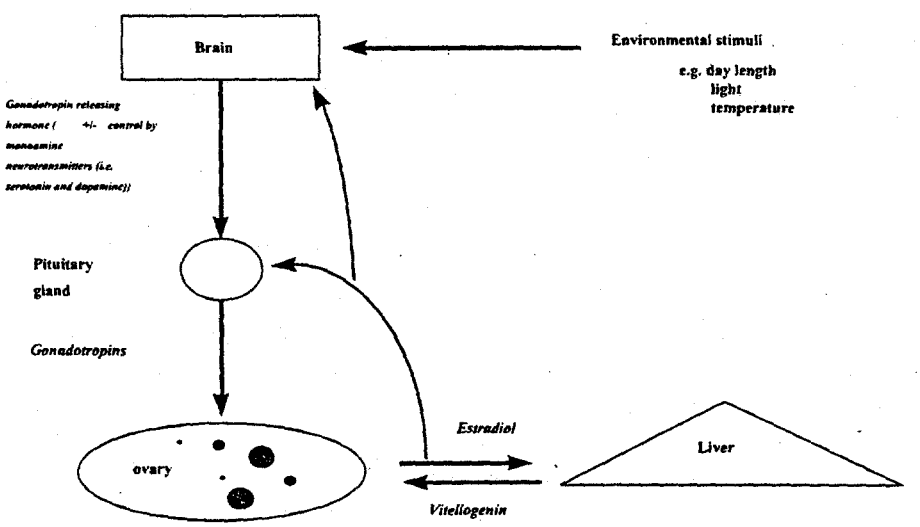

Fig. 4. Regulation of maturation in female fish (adapted from Nagahama [91] and Thomas 121]. Maturation in fish is under neuroendocrine control.

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neurotransmitters, including serotonin and through positive and negative feedback of steroid hormones [65,91]. The gonads respond by producing steroid hormones (estradiol or testosterone) that are transported via the blood to act on various target tissues. In the female, one important target tissue is the liver which contains estrogen receptors. One consequence of estrogen receptor activation in the liver is the production of vitellogenin. Vitellogenin is a phospholipoprotein, which is then transported via the blood back to the ovary, where it is broken down and incorporated into the growing oocytes as components of the egg yolk.

PCBs are known to affect this hypothalamic-pituitary-gonadal-liver (HPGL) axis at almost every point [123]. Because of its role as a storage organ (and therefore high fat content) the liver serves as a reservoir for many lipophilic chemicals including PCBs, and it is also a target for many toxic chemicals. Many of these lipophilic contaminants are incorporated into the vitellogenin and as a result, are taken up by the developing oocyte [126]. This transport of toxic chemicals into the developing oocyte is an important route of exposure for developing embryos and larvae.

2. Results of literature and data review: PCB mixtures

As discussed earlier, development of effective concentrations required, in some cases, estimating PCB concentrations in fish livers (from fish exposed in the laboratory). Thus, this section begins with a brief discussion of how liver concentrations are estimated, including a table summarizing some of the studies used to make these estimations (Table 1). Laboratory studies demonstrating the toxic effects of PCB mixtures are presented in Table 2 (effects of A1254) and Table 3 (effects of other PCBs). These tables include reproductive effects in adults and effects of PCBs on survival and development in offspring following either maternal or water exposure. The data from Table 2 are then summarized in Tables 4 and 5. Table 4 summarizes effects of A1254 on adults, and Table 5 summarizes effects of A1254 on embryo and larval survival. These two tables are used to produce estimated effective concentrations for A1254 toxicity in fish. Table 6 summarizes PCB concentration data for fish tissues (muscle, liver, and gonad or egg) reported in several different field studies. Table 7 summarizes field studies examining PCB exposure. The studies selected for inclusion in Table 7 examine sites where PCBs are one of the main contaminants (e.g. Lake Hartwell), or more commonly, sites where PCBs are associated with reproductive or developmental effects although many other contaminants are present as well.

2.1. Estimation of PCB concentrations in fish liver

Doses and routes of exposure are highly variable among laboratory studies, and can be quite different from routes of exposure in the field. One method to assess potential health effects in field animals based on laboratory studies is to convert data

Table 1
Concentration of PCBs in tissues following laboratory exposures by injection

Species	Exposure Route[a]	Dose (ppm)	Liver (ppm)	Liver to dose ratio	Reference
	PCB mixtures				
Mummichog (*Fundulus heteroclitus*)	PCB congener mix, 40 days after i.p. injection	0.76	0.75	0.98	[7]
		3.8	3.1	0.81	
		19	8.2[b]	0.43	
Mummichog	PCB mixture, 2.5 weeks after i.p. injection	1	2	2	[83]
		10	16	1.6	
		100	80	0.8	
Rainbow trout	A1254, 31 days after i.p. injection	3	0.5[c]	0.16	[85]
		30	4.0[c]	0.13	
	PCB congeners				
White perch (*Morone americana*)	3,3'4,4'-TCB i.p. 3X over 3 months, 3–6 weeks after final injection	0.025	0.03	1.2	[88]
		0.25	0.05	0.2	
		2.5	0.5	0.2	
White perch	3,3'4,4'-TCB i.p. 3X over 3 months, 3–6 week after final injection	0.6	0.5	0.83	[88]
		3	0.5	0.17	
		15	4.3	0.29	
Scup (*Stenotomus chrysops*)	3,3',4,4'-TCB (77) 12 days after i.p. injection	0.1	0.00125[c]	0.0125	[139]
		5.0	0.55[c]	0.11	
Rainbow trout (*Oncorhynchus mykiss*)	3,3',4,4'-TCB (77) 6 days after injection	0.1	0.022	0.22	[61]
		1	0.28	0.28	
		5	0.61	0.122	
Rainbow trout	3,3',4,4',5-PCB (126) 6 days after injection	0.1	0.007	0.07	[61]
		1	0.08	0.08	
		5	0.22	0.04	
European flounder (*Platichthys flesus*)	PCB 2,3,3',4,4',5-HCB (156) 8 days after s.c. injection	2.5	4.3	1.7	[108]
European flounder (*Platichthys flesus*)	PCB 2,3,3',4,4',5-HCB (156) 8 days after i.m.	0.5	13.0	26	[6]

[a] i.p. = intraperitoneal, s.c. = subcutaneous, i.m. = intramuscular injections.
[b] Values given in dry weight, liver, converted to wet weight by dividing by 4 [7].
[c] Concentrations estimated from figures.

to similar 'units,' for example the toxicity associated with PCB concentrations in a specific tissue. For the development of effective concentrations, PCB toxicity is related to concentrations in the adult liver, since the liver is a highly perfused organ, rich in lipids (resulting in accumulation of lipophilic substances), and it plays an important role during oocyte development in fish (among its many other essential functions).

In some studies concentrations are reported in several tissues, including liver and flesh or muscle. Where liver concentrations are not reported, the liver is estimated to have similar concentrations as the whole body or eggs (where those concentrations are reported). When PCB tissue concentrations are not reported at all, concen-

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trations in the liver are estimated using one of two methods. First, if the study in question used injection as the route of exposure, total dosage injected into the fish (as parts per million (ppm)) is calculated and used to estimate liver concentration. For example, if fish were dosed four times in four weeks with 25 ppm, liver concentrations are estimated to be 100 ppm. If the study in question used diet as the route of exposure, liver concentrations are estimated to be 70 percent of the total dosage received. The rational used to develop these estimates is explained in further detail below.

Table 1 summarizes several studies used to estimate PCB concentrations in the liver after injection of A1254 or individual PCB congeners. In general, concentrations in the liver will depend on uptake, distribution, metabolism and prior exposure to individual PCB congeners. Table 1 includes columns for species, route of exposure, dose, ppm in liver and the liver to dose ratio. This table also contains two sections, the first section for PCB mixtures, the second for PCB congeners. A ratio greater than one indicates accumulation in the liver to concentrations greater than the injected dosage. A ratio less than one indicates liver concentrations less than the injected dosage. Since there is very little consistency among the studies included in this table (e.g., the route and duration of exposure and type of PCB used for each study is different), it is not possible to produce a mean liver/dose ratio. The ratios reported in this table range from a low of 0.01 to a high of 26. The ratio reflects the culmination of several different processes including distribution, metabolism and depuration. The degree to which these processes occur depend on many factors including the PCB mix or congener, when the analysis occurred (days to weeks or months after injection), route of exposure, initial dose, species and sex.

The most useful studies in this table for determining liver concentrations following injection of A1254 are those that injected PCB mixtures rather than individual congeners. Three PCB mixture studies are listed in Table 1. The ratios in this section are calculated for studies by Black [7], McElroy et al. [83], and Melancon et al. [85], and range from 0.13 to 2. Black [7] injected (i.p.) a mixture of mono-and co-planar PCBs into *Fundulus heteroclitus* and measured liver concentrations after 40 days. The calculated liver/dose ratios from this study decrease with increasing PCB dose (ratios are 0.98 to 0.43 following initial doses ranging from 0.76 to 19 ppm, respectively). McElroy et al., [83] injected (i.p.) a PCB mixture (1:1.5 A1262:A1248) into Fundulus heteroclitus and measured liver concentrations after 18 days. Similar to Black [7] the calculated ratios decrease with increasing dose (ratios are from 2 to 0.8 following doses ranging from 1 to 100 ppm). In this case, the lower doses of 1 and 10 ppm produce liver concentrations that are higher than the injected dose (i.e., liver/dose ratios greater than one). Finally the study by Melancon et al., [85] (who injected A1254) showed the same trend (a smaller ratio with higher PCB dose) but both ratios were very low (0.16 and 0.13), indicating much lower concentrations of A1254 in the liver than the injected dose, 31 days after injection.

Liver concentrations are estimated for two studies in which fish were injected with 25 ppm of A1254 (i.p) once a week for four weeks (a total of 100 ppm)

Table 2
Reproductive and developmental effects of laboratory exposure to A1254

Species	Dose (ppm)	Tissue Concentration[a]	Effects	References
		Effects in adults		
Carp	25x4 (i.p.) over 4 week period	NR (est 100 in liver)	↓ Androgen by 61 %[b], ↓ estrogen by 35 %, ↓ corticosteroids by 35 %, abnormal spermatozoa	[112,113]
Trout	25x4 (i.p.) over 4 week period	NR (est 100 in liver)	↓ Androgen by 45 %, ↓ estrogen by 33 %, ↓ corticosteroids by 44 %, abnormal spermatozoa	[112,113]
Atlantic croaker	3.4 in diet for 30 days.	NR (est 71 in liver)	↓ Ovarian growth by 75 %, ↓ Testosterone, no effect on estradiol.	[125]
Atlantic croaker	5 in diet for 17 days.	NR (est 60 in liver[c])	↓ Ovarian growth (GSI) by 50 %, ↓ estradiol by 49 %, ↓ GtH secretion by 54 %, ↓ Plasma vitellogenin, ↓ hepatic estrogen receptor concentration	[124]
Atlantic croaker	1 in diet for 30 days	13.0 brain 2.4 testes 25.4 liver	↓ serotonin in preoptic-anterior hypothalamic area (POAH) by 38 %, ↓ dopamine in POAH by 35 %, ↓ serotonin in medial and posterior hypothalamus (MPH) by 38 %, ↓ dopamine in MPH by 29 % ↓ Testicular growth Inhibition of GtH secretion, ↓ Testiscular GSI by ~79 %, ↓ Testosterone by ~60 %, ↓ 11-ketotestosterone by ~73 %	[67,68][d]
Brook trout	0.2 in water for 21 days	77.9 in eggs 32.8 in skeletal muscle (est 78 in liver)	↓ Testes size, ↓ Spermatic fluid[e]	[38]
Atlantic cod	1, 5, 10, 25, 50 in diet for 5 months	45–374 (in liver)	Altered steroid production, Abnormal testes with all doses, ↓ Spermatogenic elements.	[109]
Rainbow trout (juvenile)	3, 30, 300 in diet for 6 months	NE[f]	↓ E2 induced VTG production by 60–72 %.	[18]
		Effects in embryos and larvae		
Lake trout	269 ng/L in water	NE[f]	No effect on fertilization	[37]

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Table 2 (continued)
Reproductive and developmental effects of laboratory exposure to A1254

Species	Dose (ppm)	Tissue Concentration[a]	Effects	References
Atlantic croaker	Adults exposed to 1 in diet for 30 days	NR (est 30 in whole body)	↑% Abnormal embryos by 33 %, ↓Hatch by 14 %, ↓Viable hatch by 32 %, ↓Larval length	[111]
Brook trout	Adults exposed to 0.2 in water for 21 days[e]	77.9 in eggs	↓ Percent hatch by 20 %[e]	[38]
Brook trout	Eggs exposed to 0.43–13 ug/L in water from 10 days before hatch to 118 days post hatch	17	↓ Backbone phosphorus by 38 %, ↓ hydroxyproline by 20 %,	[81]
		71	↑ Backbone calcium by 100 %.	
		125–284 (fry, whole body)	↓ Fry survival by 21–50 % (no effect on egg hatch)	
Coho salmon	Eggs exposed to 5–80 ug/L in water from 2 weeks before hatch to 4 weeks post hatch	NR		[50]
		10 ug/L and higher	Premature hatch by 2–5 days, ↓ Hatch rate by 18–34 %, ↓ Fry survival by 30–93 %	
Sheepshead minnow	Adults exposed for four weeks to 0.1–10.0 ug/L A1254 in water	0.88	No effect	[52]
		5.1–170 (in eggs)	↓ Percent fry survival 1 week post-hatch by 20–100 %	
Sheepshead minnow	Adults and offspring exposed for 3 weeks 0.1–10.0 ug/L A1254 in water	0.88	no effect	[110]
		5.1–170 (est in eggs)	↓ Percent fry survival 2 weeks post-hatch by 30–90 %	

[a] Where reported, tissue concentrations are in parts per million (ppm) wet weight. Where no concentrations are reported, they are estimated in the liver (est. liver) by calculating total exposure to the fish for injection (i.p.) or 70 % of dose for oral exposure.

[b] Percent change from controls are calculated using reported mean values (e.g., percent hatch or percent survival, or plasma concentrations of steroid hormones)

[c] Ungerer and Thomas [126] report concentrations of 99 ppm and 55 ppm in female and male livers respectively after 30 days of feeding 5 ppm A1254.

[d] The same set of fish were used for both studies [67,68] (Izhar Khan, personal communication, University of Texas, Austin), PCB concentrations reported in [67] apply to [68] as well.

[e] Corexit 7664 was used as a solvent for these studies. Fish exposed to Corexit only appeared to have fimer livers and discolored testes compared to control fish. Statistics were not reported for testicular data. Hatch was reduced from 92 % to 72 %. Statistics were not shown for this figure, nor were hatch results for eggs exposed to Corexit only.

[f] I could not estimate liver concentrations for these species because there was insufficient information (i.e., no other tissue concentration reported, no information on daily intake of PCB contaminated food).

[112,113]. Based on the data in Table 1, I chose to use a ratio of 1 to estimate A1254 concentration in liver tissue for these studies. This value was selected based on a consideration of the dose used (100 ppm, similar to McElroy et al.,[83]), and the length of exposure (total exposure was four weeks, but injections were given weekly, so that fish were killed within one week of the last injection, and four weeks after the first injection). The one study with A1254 by Melancon et al., [85] resulted in liver/dose ratios much lower than 1, however these authors used lower PCB concentrations and measured A1254 in tissues after a much longer time period (31 days). This factor is most likely to overestimate the concentration of A1254 in the liver. As is clear from the data in Table 1, however, the uncertainty associated with this estimate could vary within an order of magnitude.

Several other studies in Table 1 report liver concentrations following injections of PCB congeners. These studies are relevant to the PCB congener section of this report. It is not surprising that the liver concentrations (and therefore liver/dose ratios) vary significantly with PCB congener because there can be large differences in half-life due to distribution, metabolism and excretion [96,97]. Table 1 reflects these differences, as liver/dose ratios of congeners range from 0.01 to 26. Almost all ratios greater than one were calculated using data from Beyer et al. [6] and Sandvik et al. [108]. Both of these studies used mono-ortho hexachloro-PCB 156, but employed different injection techniques. These data suggest that this compound is accumulated in the liver, is not well metabolized, and that final concentrations can vary depending on the route of exposure. In contrast, liver ratios of PCB 77 ranged from 0.013 to 1.2 of the total injected dose. It is notable that the only ratio above one was for the smallest total dose of 0.025 ppm (which is a very low dose for this congener). Ratios of less than one correspond to total doses ranging from 0.25 to 15 ppm. The low ratios for this congener are explained by the relatively rapid elimination of both metabolized and unmetabolized PCB 77 in fish [139].

In the congener section of this review, liver concentrations of PCB 77 are estimated for several studies. The doses used in these studies range from 0.2 to 5 ppm. Based on these concentrations and the liver/dose ratios in Table 1, I use a liver/dose ratio of 0.3 to estimate liver concentrations. This will most likely result in an overestimate of concentrations of PCB 77 in liver tissue. As with the A1254 estimate, liver concentration estimates for a given dosage can vary within an order of magnitude.

When fish are exposed via the diet, several groups report that approximately 30 % to 70 % uptake occurs for PCB mixtures [57,75], although assimilation can vary from less than 20 % to more than 80 % for individual PCB congeners [15,22,23,35,96,97]. Liver PCB concentrations are estimated for two feeding studies conducted by Thomas [124,125] using A1254. Based on the studies cited above, the fish in these studies are estimated to have received 70 % of the total dose. As with the injection estimate, this estimate is more likely to overestimate PCB concentrations because it assumes efficient assimilation for almost all PCB congeners.

It is notable that PCB accumulation and distribution in fish tissues is highly influenced by the lipid content and lipid type (polar, neutral, nonpolar). Thus, accumula-

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tion and deposition of PCBs into tissues including developing oocytes can be influenced by the percent and type of lipids in the adult fish and in the oocytes [66,95]. This will likely contribute some uncertainty to the PCB concentrations estimated in this analysis, since all species are treated similarly.

2.2. Laboratory studies: PCB mixtures

The reproductive and developmental effects of PCBs in fish following laboratory exposure are listed in Tables 2 and 3. The categories in Tables 2 and 3 include species, dose (ppm), tissue concentration (ppm), effects and references. Effects of A1254 in adult animals are presented in the top portion of Table 2, followed by effects in embryos and larvae. The common names for each species are listed under species. Dose is summarized as amount (in ppm), route, frequency and total exposure period. For example "25 × 4 (i.p) over 4 week period" indicates that fish were exposed to four 25 ppm injections over a four week period. "Tissue concentrations" refers to either the reported or estimated tissue concentrations. If concentrations are not reported in any tissue (NR), liver concentrations are estimated as described above. Wherever possible, effects are summarized as a percent change from control using arrows to indicate a reduction or increase. If values are not reported as percent change from control, this change is calculated using the mean values provided in the reports. The last column of Tables 2 and 3 provides the references for each summarized study. Table 3 summarizes studies evaluating the effects of PCB mixtures other than A1254 on reproduction and development. Because the toxicity of PCB mixtures varies with the degree of chlorination, there is a broad range of concentrations associated with effects, and in some cases the mixtures did not affect the endpoints used in these studies (e.g., PCB 1016 [51]). In general, exposure to PCB mixtures resulted in altered reproduction and reduced embryo and larval survival.

Tables 4 and 5 are constructed to aid the development of effective concentrations for A1254 toxicity. Table 4 is a summary of the data on adult fish presented in Table 2, and Table 5 is a summary of the data on embryo and larval fish presented in Table 2. Included in the tables are: concentration of A1254 in liver (reported or estimated) for Table 4, and whole body for Table 5, effects of exposure and references. When developing 'effective concentrations' for A1254 it was important to consider some of the following qualities of the studies listed in Table 2: 1) concentrations used in the study (e.g. do they result in environmentally relevant concentrations); 2) consistency of effects among studies that evaluated similar endpoints (i.e., reproducibility); 3) validity of controls; and 4) amount of information provided (statistics, chemical analysis, number of fish used, amount of data presented).

Several studies included in Table 2 are not only consistent with each other [67,68,124,125], but are consistent with results reported by other researchers (e.g 38,112,113). In addition, although the concentrations used in these studies are high, they are environmentally relevant for sites such as the Hudson River or New Bed-

Table 3
Reproductive and Developmental Effects of PCBs other than A1254

Species	Dose (ppm)	Tissue Concentration	Effects	References
Stickleback (adult)	Clophen A50 in diet for 3.5 months	102–289 (whole body, wet weight)	Trend toward ↓ percent successful spawn (from 80 % in control to 20–55 % in PCB exposed fish)	[57]
Minnow	Clophen A50, 20, 200, 2000 in diet 40 days	1.6 15 170 (whole body wet wt)	No effect. Spawn delayed 7 days. Spawn delayed 21 days, ↓ Percent hatch by 82 %, ↑ Mortality	[5]
Dab	Clophen A40 14 and 26 in diet, three times over four months	0.2–0.6 in eggs	No effect in egg production, quality, fertilization, hatch or survival	[36]
Atlantic salmon	A1016, 1221, 1254, 1260 mixture in water for 48 hour, health assessed over 6 months	0.85–1.53 [a] 5.59 14.16 (concentrations for embryos)	No effect Reduced wet weight and length after 6 months Reduced weight and length, altered behavior, reduced predator avoidance	[34]
Rainbow trout	1,5 and 20 A1260 in the water (mg/L) for 2 hours at 25 d post-hatch	2.1 2.5	Increased proportion of female offspring Increased percent females with incomplete or inconsistent oocyte development	[80]
Goldfish	A1248 250 i.p. injection	250 (estimated)	Stimulated metabolism of progesterone, estradiol, testosterone and cortisol, decreased plasma concentrations of estradiol, testosterone and progesterone	[79][d]
Coho salmon	A1254 and A1242 in 4:1 ratio. 50 and 500 in feed for 2 or 3 months	Low dose High dose	No effect Reduced serum T3 levels, no effect on T4, increased T4/T3 ratio, reduced body weight	[74]
Sheepshead minnow	0.1–10 ug/L A1016 in water for 28 days	4.2–66 (in eggs)	No effect on fertilization, No effect on hatching, No effect on fry survival	[51]
Fathead minnow	0.1–2.1 ug/L A1260 from embryo to spawning adult (250 days)	360 in whole body of adult males	No effect on reproduction, including hatching of offspring.	[24]
Fathead minnow	1.3–9 ug/L A1260 for 30 days post-hatch	≥ 4 ug/L [b]	↓ Survival by 69 %, weight by 30 % and length by 11 %.	[24]

11.1335

Table 3 (continued)
Reproductive and Developmental Effects of PCBs other than A1254

Species	Dose (ppm)	Tissue Concentration	Effects	References
Fathead minnow	1.1–8.5 ug/L A1248 for 30 dayspost-hatch	≥ 4.4 ug/L[b]	↓ Survival by 44 %, weight by 66 % and length by 30 %.	[24]
Fathead minnow	0.1–3.0 ug/L A1248 from embryo to spawning adult (250 days)	190 in whole body of adult males	No effect on reproduction, including hatch of offspring.	[24]
		Mixtures of individual congeners		
Zebrafish	41, 51, 60, 68, 91, 99, 104, 112, 115, 126, 143, 153, 169, 184, 193, 0.008, 0.08, 0.4 ppm of each congener in food, sampled at 4 and 13 weeks	Sum PCB concentrations at 13 weeks:		[98]
		0.14	no effect on mortality, ↓ body weight[c] ↓ liver somatic index (LSI)	
		1.1	↑ mortality by 7 %, ↓ body weight[c] ↓ GSI, ↓ mature oocytes, ↓ LSI	
		2.7 (in whole body with liver and ovaries removed)	↑ mortality by 17 %[c], ↓ body weight[c] ↓ mature oocytes[c], ↓ LSI, ↓ larval survival	
Fundulus heteroclitus	118, 105, 167, 156, 157, 189, 77, 126 (mixed together) 0.76, 3.8, 19 of PCB mixture (i.p.)	2.9 12.2	none ↓ egg deposition by 63 %, ↓ food consumption, ↑ female mortality	[8]
		32.8 (liver dry wt after 40 d)	↓ egg deposition 60 %, ↓ pituitary gonadotropin, 58 % adult mortality, ↓ food consumption	

[a] These concentrations are measured in embryos; health effects are measured in older fry with lower PCB concentrations (as a result of dilution during growth).

[b] Tissue concentrations not reported, unable to estimate concentrations in larvae from data provided.

[c] Weight loss due mainly to reduced gonad growth.

[d] Abstract only, published in Japanese.

ford Harbor. Together these studies provide strong evidence for adverse reproductive effects of A1254 in adult fish beginning at 25 ppm in the liver.

The effects of A1254 on embryo and larval growth and survival are reported in several studies. Two of these studies use a water exposure [50,81] and two use maternal exposures 52,109]. Concentrations in the larvae are either provided or could

11.1336

Table 4
Estimation of A1254 effective concentrations in fish from laboratory studies[a]

Concentration of A1254 in liver (ppm)	Effects	References
25	↓ Serotonin, ↓ dopamine, ↓ testicular growth by 79 %, ↓ T and 11-KT, inhibition of GtH secretion *in vitro*	[67,68]
60 (estimated)	↓ Ovarian growth by 50 %	[124]
71 (estimated)	↓ Ovarian growth by 75 %, ↓ testosterone	[125]
45–100	Abnormal testes, altered steroid hormone metabolism, ↓ spermatogenic elements	[109]
90 (estimated)	↓ Decrease sperm fluid, ↓ testicular growth	[38]
100 (estimated)	↓ Androgens, estrogens, corticosteroids, abnormal spermatozoa	[112,113]

[a] Chen et al. 1986 is not included since concentrations could not be reliably estimated.

be estimated, except for the studies by Halter and Johnson [50] and Foster and Berlin [37] (there is insufficient data available for estimating concentrations in larvae following water exposures to the eggs). In the study by Sharp and Thomas (reported as a meeting abstract only [111]), concentrations in larvae are estimated as less than 30 ppm since the total dose in the adults is 30 ppm. There was a wide range of A1254 concentrations associated with larval mortality and or reduced growth (from 5–125 ppm estimated or measured in whole bodies). It is important to note that there may be sublethal effects other than reduced growth that were not measured in these studies that can affect larval survival in the wild.

2.3. Estimation of effective concentrations for adults and larvae: A1254

The studies summarized in Table 4 demonstrate that A1254 exposure resulting in approximately 25 to 100 ppm PCB in the liver of adult fish consistently causes reduced gonadal growth and altered blood plasma steroid hormone concentrations. Fry survival, as demonstrated by the studies summarized in Table 5, is decreased by PCB concentrations ranging from 5 to 125 ppm. It is possible that the wide range is a result of either different routes of exposure (water exposure to eggs versus maternal exposure), assay temperature differences (12 to 14 °C for the brook trout versus 25 to 30 °C for the sheepshead minnows), or interspecies differences in egg and larval development or sensitivity to PCBs.

The effective concentrations for reproductive and developmental effects of A1254 in fish are summarized in Fig. 5, and are shown in comparison to concentrations of A1254 recently measured or estimated in fish from the Hudson River (NY), New Bedford Harbor (MA) and Lake Hartwell (SC/GA). At all of these sites, PCB

11.1337

Table 5
Estimation of A1254 effective concentrations in fish from laboratory studies: effects in embryos and larvae[a]

Concentration of A1254 in whole body (ppm)	Effects	References
5 (estimated)	↓ Fry survival at 1 week post-hatch	[52]
5 (estimated)	↓ Fry survival at 2 week post-hatch	[110]
17	↓ Backbone collagen	[81]
< 30 (estimated)	↓ Percent hatch, larval length ↑ abnormal embryos	[111]
< 71	↓ Fry weight	[81]
125	↓ Fry survival	[81]

[a] Halter and Johnson [50] and Foster and Berlin [37] are not used in this table since concentrations in eggs or fry could not be reliably estimated.

concentrations in fish tissues are reported as 'total PCB' rather than as any specific mixture such as A1254. It is most relevant to compare the concentrations A1254 shown to cause the effects reported in laboratory studies to concentrations of A1254 measured or estimated in fish exposed in the field. Thus, concentrations of A1254 are estimated for the most highly contaminated fish from these four sites, using the proportion of total PCBs quantified as A1254 as reported or communicated for each of these sites as described below.

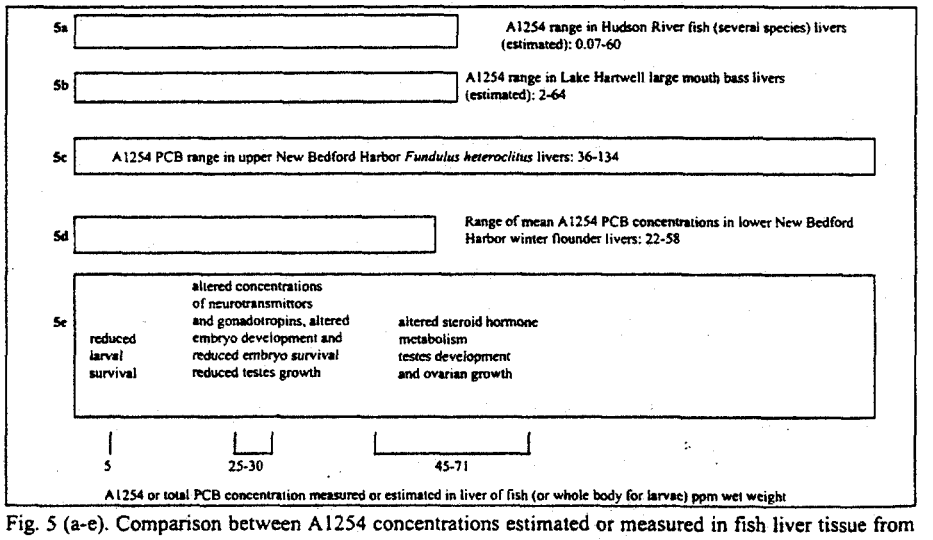

Fig. 5 (a-e). Comparison between A1254 concentrations estimated or measured in fish liver tissue from the Hudson River (NY) [84], Lake Hartwell (SC/GA) ([40]; Mark Greeley, personal communication, Oak Ridge National Laboratory, Oak Ridge, TN), and New Bedford Harbor (MA) [31]; Rick McKinney, personal communication, US EPA, Atlantic Ecology Laboratory, RI) and tissue concentrations of A1254 shown to cause reproductive and developmental effects in laboratory studies.

11.1338

The total PCB levels for Hudson River fish were measured in standard fillets, where essentially one side of the fish is intact and includes the ribs, skin and belly-flap. In 1997, total PCB concentrations ranged from 0.21 to 57.4 ppm wet weight [114]. Although A1254 and A1260 were both quantified, concentrations of the more highly chlorinated PCB mix were reported simply as 'A1254 and above'. In the Hudson River both the proportion reported as A1254 and above, and the proportion measured as A1260 decrease with increasing river mile (Ronald Sloan, New York State Department of Environmental Conservation, Albany, NY, personal communication). Thus, the proportion of PCBs quantified as 'A1254 and A1260' range from 88 % at RM 11 to 56 % at RM 189 [114]. Of the PCBs quantified as A1254 and A1260, the proportion of A1260 ranges from 41.2 % at RM 40 to 5.4 % at RM 189 (Ronald Sloan, pers comm). Using these figures, fillets from the most highly contaminated fish at RM 189 (reported to have total PCBs of 57.4 ppm), are estimated to contain 30 ppm of A1254.

Concentrations for Lake Hartwell were also measured in fillets with skin on one side. Percentage of total PCB that is A1254 ranges from 66–95 % for individual fish collected at the same site (Mark S. Greeley, personal communication, Oak Ridge National Laboratory, Oak Ridge, TN). Concentrations in the flesh ranged from 2–48 ppm total PCB for fish collected in 1990 and 1992 (concentrations were lower in 1990 than in 1992) [40]. Using the lower end of the A1254 range of 66 %, A1254 concentrations in the fillets are estimated to range from 1–32 ppm. Finally, the concentrations of A1254 in the fillets are converted to concentrations in the liver so that A1254 concentrations in these fish can be compared to the 'effective concentrations' developed for A1254. To convert from concentrations in fillets to concentrations likely to be measured in the liver of Hudson River and Lake Hartwell fish these values are doubled (as described below) so that the estimated liver concentrations range from 0.07–60 ppm in the Hudson River and from 2–64 ppm in Lake Hartwell (Fig. 5a and 5b).

This estimation of liver PCB values (twice the concentration found in the flesh) is based on previous studies in which PCB concentrations are reported for various tissues for wild-caught fish (Table 6). Field studies were selected for this table since PCB concentrations are being extrapolated from muscle to liver in wild-caught fish and tissue distribution may vary depending on the type of exposure (i.e., a relatively "acute" laboratory exposure vs. a chronic field exposure). The first two columns in Table 6 list the site, species and PCB mixture reported in each study. The next three columns include tissue concentrations (in ppm) for muscle, liver and ovary. The next column lists the liver/muscle ratio. The liver/muscle ratios range from <1 to 77, with the majority of studies ranging from 1 to 8. Variations in ratios are the result of several factors including sex, reproductive status, interspecies differences in lipid content, metabolism, excretion, life history, and even analytical techniques (e.g., skinless fillet vs. skin-on fillet). However, as reflected in the table, concentrations of PCBs in liver are often more than double that of muscle tissue (only 2 out of 10 ratios are below 2). This two-fold liver concentration estimate was selected since

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Table 6
Concentrations of PCBs in various tissues reported in field studies

Species	PCB	Muscle (ppm)	Liver (ppm)	Ovary (o) or gonad (g) (ppm)	Liver/ muscle	Ovary or gonad/muscle	References
Baltic herring	PCB mix	0.15–1.8	0.02–0.38	0.02–0.24 (o)	<1	0.13	[53]
Fundulus heteroclitus, Hudson River (Piermont)	PCB mix	0.24	1.25	3.33 (o)	5	14	[83]
Fundulus heteroclitus, Hudson River (Newark)	PCB mix	0.20	1.65	2.01 (o)	8	10	[83]
Atlantic cod	A1254	<0.000002–0.002	0.154	0.005 (o)	77	2	[55]
Red mullet, Western Med	PCB mix	0.307	1.093	1.413 (g)	4	5	[3]
Horse mackerel, Western Med	PCB mix	0.127	0.357	4.575 (g)	3	38	[3]
Blue whiting, Western Med	PCB mix	0.034	0.040	0.32 (g)	1	9	[3]
Striped bass Shubenacadie River	PCB mix	0.01	NA	0.04	NA	4	[105]
Striped bass Annapolis River	PCB mix	0.02	NA	1.4	NA	70	[105]
Pike, southern Scandinavia	PCB mix	0.0065–0.014	NA	0.49–7.63	NA	75–545	[72]
Dab, Eastern Channel, France	PCB 138	0.23	0.40	0.13	2	0.6	[76]
	PCB 153	0.21	0.44	0.97	2	5	

fillets or muscle tissue samples for the Hudson River and Lake Hartwell fish were analyzed as 'skin-on' fillet, which may result in higher concentrations of PCBs compared to skinless fillets.

PCB concentrations are also available for fish from New Bedford Harbor, MA. PCBs were measured in liver tissue from both *Fundulus heteroclitus* and winter flounder collected from two sites in New Bedford Harbor. In the upper harbor, where *Fundulus* were collected from a 'hot spot', A1254 comprises approximately 40 % of total PCBs measured in that location (Rick McKinney, personal communication, US EPA, NHEERL, Atlantic Ecology Division, Narragansett, RI). Concentrations of A1254 in *Fundulus heteroclitus* from the upper harbor range from 36–134 ppm wet weight (Fig. 5c) (Rick Mckinney, unpulished data). Winter flounder were collected from the lower harbor where A1254 comprises approximately 70–80 % of total PCBs measured. Mean concentrations of A1254 in winter flounder from the lower harbor were 38 ppm wet weight (using a wet:dry ratio of 4:1 (A. El-

11.1340

Table 7
Reproductive and Developmental Effects Associated with PCB Exposure in the Field

Site	Species	Contaminants at site	Concentration Range[a] ppm wet wt	Observation Associated with PCB	References
			Effects in adults		
Hartwell Lake, SC/GA	Large mouth bass	PCBs	4–20 in flesh	↓ Liver estrogen receptor binding, ↑ nonspecific binding ↓ Testosterone, ↓ White blood cell counts, ↑ DNA repair, ↓ Ovary size.	[1,39,127]
New Bedford Harbor, MA	Winter flounder	PCB Others	2–47 in liver	↓ Ovary size in 1 out of 3 years[b]	[29]
Puget Sound (Duwamish Waterway)	English sole	PCB PAH Others	~10 in liver ~ 1 in ovary	↑ Fecundity ↓ Egg size ↓ Vitellogenin[c]	[64]
Oak Ridge, TN	Redbreast sunfish	PCB, PAH, metals, Others	NR	↓ Fecundity (egg clutch size)	[1,2]
Rotterdam Harbor (dredge material)	Flounder	28, 52, 101, 118, 153, 180 Other PCBs and chemicals	Sum: 11.9 (in liver) ppm lipid	Induced premature vitellogenesis	[62]
			Effects in embryos and larvae		
Baltic Sea	Baltic herring	PCB DDE PAH Others	0.24 0.02 in ovary	↓ Viable hatch ↓ Larval survival	[53]
Lake Geneva	Arctic char	PCB DDT Others	0.10–0.31 0.04–0.17 in eggs	↓ Embryo survival ↓ Fertility	[86]
San Francisco Bay	Starry flounder	PCB Others	5–30 lipid (~50–200 in eggs)	↓ Embryo survival	[116]
Lake Michigan	Lake Trout	PCB Others	0.25– 7.76 in eggs 4.19–13.88 in testes	↓ Embryo survival	[77]
Lake Michigan	Chinook salmon	PCB Toxaphene Others	2.83–9.09 2.37–5.93 in eggs	Negative correlation with survival to "swim up"	[41]
Baltic Sea	Baltic flounder	PCB Others	0.12 in ovary	↓ Embryo survival, ↓ Larval survival	[132]
New Bedford Harbor, MA	Winter flounder	PCB Others	39.6 in eggs	↓ Larval length at hatch, ↓ Larval weight at hatch	[10]

11.1341

Table 7 (continued)
Reproductive and Developmental Effects Associated with PCB Exposure in the Field

Site	Species	Contaminants at site	Concentration Range[a] ppm wet wt	Observation Associated with PCB	References
New York	Striped bass	PCB DDT HCB chlordanes Others	7-32 in eggs	slightly reduced larval survival	[138]
Puget Sound (Urban areas)	English sole	PCB PAH Others	2.6, 3.5 in adult livers from urban areas	↓ Larval survival	[17]
New Bedford Harbor	mummichog	PCB (mono- and non-ortho) Other chemicals	0.4–29 liver, dry wt.	↑ embryo mortality ↑ terata ↑ adult mortality	[9]
River Skalice, Czech Republic	Carp	28, 52, 101,153, 138, 180, 118	Sum: 0.206 wet wt. (in eggs) 0.262 wet wt. (in liver)	↑ length growth rate ↓ weight condition	[122]

[a] Concentrations associated with observation, concentration ranges or means are provided when no attempt was made to calculate an effect level.
[b] Reduced ovary size was observed in the first year of the study. Sample size decreased after first year.
[c] Reduced plasma VTG concentrations were associated with PCBs but were not reduced in Duwamish fish.

skus, personal communication, SUNY Stony Brook, Stony Brook, New York)) (Fig. 5d) [31]. One individual not included in this mean, had liver concentrations of approximately 183 ppm A1254 wet weight, inclusion of this individual would result in a mean of 58 ppm [31].

Adverse effects on reproduction or development are summarized in Fig. 5e. As illustrated in Fig. 5, A1254 concentrations in fish from all three sites overlap A1254 concentrations shown to adversely affect reproduction in adult fish in laboratory studies. In general, there is little information relating A1254 concentrations in adult fish to A1254 concentrations in their offspring, thus it is difficult to estimate A1254 concentrations in eggs or larvae from these sites. However, several studies have reported similar or higher PCB concentrations in eggs compared to adult muscle tissue (Table 6). The egg or gonad to muscle ratios range from 0.1 to 545. The majority of the calculated ratios, however indicate at least five times greater concentrations of PCBs in the eggs compared to muscle tissue. It is possible, therefore, that A1254 concentrations in eggs in some fish from the Hudson River, Hartwell Lake and New Bedford Harbor may also be well within the range of A1254 concentrations (from 5–125 ppm) found to be toxic to embryos or larvae in laboratory studies.

2.4. Field studies: PCB mixtures

Table 7 summarizes studies demonstrating an association of PCB mixtures in the field with reproductive and developmental effects. The categories in Table 7 are site, species, contaminants at the site, concentration range, observations associated with PCBs, and references. In most cases, sites are contaminated with chemical mixtures. To date, there is only one site contaminated almost exclusively by PCBs for which data are available (Hartwell Lake, SC/GA). Concentrations reported in Table 7 are either the mean concentration associated with a particular effect or the concentration ranges reported at sites where effects are associated with PCB exposure.

Three studies in Table 7 report an association between PCB exposure in the field and adverse effects in adult fish, although concentrations of PCBs associated with effects are published in only two of the studies. In Lake Hartwell, reduced estrogen receptor binding [39], reduced testosterone concentration, reduced ovary size and a trend toward greater damage in oocytes are associated with tissue concentrations as high as 20 ppm in the flesh (which could be estimated at 40 ppm in the liver) [1,127]. This site is known to be contaminated primarily with PCBs, and other chemicals are reported to be too low in concentration to be of concern. The authors of one of the Lake Hartwell investigations conclude that there appears to be a "direct relationship between the health of fish in Lake Hartwell and PCB contamination" [127]. Reduced ovary size [29,30] is associated with similar concentrations of PCBs in fish from New Bedford Harbor (as high as 47 ppm in the liver). The authors report this finding in the first year of a three year study. However, they also report much smaller sample sizes after the first year (Adria Elskus, pers comm). Although PCBs are the main contaminant of concern in New Bedford Harbor, other important contaminants are known to be present as well. In general, the PCB concentrations associated with effects on steroid hormone concentration, estrogen receptor binding, and ovary size (approximately 40 ppm in the liver) are within the range of those concentrations found to have similar effects in laboratory studies (Table 2; Fig. 5).

The remaining studies in Table 7 focus on the association between PCBs and embryo and larval survival. There are nine studies included in this section of the table. These studies are from sites around the world and across the United States. Although effects are associated with PCB contamination, other contaminants are acknowledged to be present at all of these sites. Reduced embryo survival is associated with PCB concentrations ranging from 0.1 to 200 ppm in the eggs. Three different studies indicate that PCB concentrations as low as 0.1 to 8 ppm could reduce embryo survival [77,86,132], and one reports reduced survival with concentrations of approximately 50 to 200 ppm in the eggs (measured as 5 to 30 ppm in the lipid) [116]. Reduced larval survival or viable hatch (the production of apparently healthy larvae) is reported in four field studies in association with PCB concentrations ranging from 0.1 to 32 ppm in eggs or ovaries [53,132,138]. None of these studies reported any pathology associated with the larval mortality, precluding a comparison

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to pathological effects caused by PCBs in laboratory studies. A more sublethal response, reduced size (weight and length) is reported in larvae hatched from eggs with PCB concentrations of 39 ppm [10]. PCB concentrations from 5 to 125 ppm (eggs or larvae) reduce larval survival in laboratory studies (Table 2; Fig. 5). These values are within range of the PCB concentrations associated with reduced survival in the field.

A few field studies report an association with very low (0.1 ppm) concentrations of PCBs. It is important to consider the presence of other contaminants at most of these field sites. For example, the two sites reporting an association between larval survival and low concentrations of PCBs [53,86] also report that other contaminants that are toxic to embryos and larvae, including DDT (or DDE), are present at these sites. Some sites contain chemicals such as dioxins and polyaromatic compounds in addition to PCBs that can all act via the same mechanism. The effects of these chemicals may be additive, synergistic or antagonistic. One consequence of such mixtures may be that the concentration of any single class of chemicals (e.g., PCBs) associated with toxic endpoints in the field may be lower than the concentration required to cause toxicity in the laboratory, if laboratory exposure is limited to a single compound. In addition, there are many 'natural' variables (e.g., temperature fluctuation) which may interact with chemical contaminants and influence embryo and larval mortality [131].

2.5. Discussion and conclusion: PCB mixtures

The data summarized in Fig. 5 indicate that adult concentrations of A1254 as low as 25 ppm in the liver may affect the functioning of the hypothalamic-pituitary-gonadal-liver axis (HPGL). Altered function of the HPGL may affect reproductive success by altering gonad growth, including oocyte development and maturation, and spawning (e.g., number of eggs spawned, delayed spawning, inhibited spawning). Any one of these results may eventually affect recruitment. In addition, several studies have shown that embryos and larvae are even more sensitive to A1254 than adult fish. It should be noted that the endpoint used in embryo and larval studies is often reduced survival. This suggests that there may be sublethal effects at even lower concentrations than those found to cause embryo or larval toxicity (such as growth or behavior). Laboratory studies have shown embryo or larval survival to be affected by concentrations as low as 5 ppm. The concentrations of A1254 shown to be toxic in the laboratory are within range of the A1254 concentrations estimated in some of the most contaminated fish from the Hudson River, Lake Hartwell and New Bedford Harbor.

There are a number of field studies at other sites around the world that demonstrate an association between PCB contamination and altered reproductive success. In the majority of these sites there are other contaminants present that may co-occur with PCBs, including metals, DDT (and metabolites), and polycyclic aromatic hydrocarbons. All of these contaminants are known to affect reproduction in fish [70,123], making comparisons between PCB concentrations found to be toxic in the

laboratory (a single chemical exposure) and those associated with altered reproductive success in the field (most often a mixed chemical exposure) difficult. However, there is a fair amount of agreement between the concentrations and effects from laboratory studies and field studies, suggesting that A1254 exposure at concentrations from approximately 25 ppm and higher in adults livers, and 5 ppm and higher in embryos and larvae, can affect reproduction and development in fish.

Several recent studies have demonstrated acquired resistance to AhR-active chemicals in two species, *Fundulus heteroclitus* (mummichog) [4,32,103,104,130] and the Atlantic tomcod [140]. Resistance has been demonstrated at the biochemical level (e.g., resistance to CYP1A induction: [4,32,130,140] or as reduced mortality [60,90,103,104]). Currently the only species for which acquired resistance is known to affect embryo and larval survival following exposure to AhR-active chemicals is *Fundulus heteroclitus* (reviewed in Hahn [47]). Although Fundulus adults (and second generation offspring from adults reared in the laboratory) from New Bedford Harbor are more resistant to the toxic effects of AhR compounds when exposed in the laboratory [8,9,90], Black et al. [8,9] reported higher incidence of terata and a reduced survival rate in first generation offspring from non-depurated NBH adults when reared in the laboratory, compared to offspring from reference sites. Other studies with the first generation offspring of this population have shown that larval viability is negatively correlated with female size and presumably age (T. Gleason, personal communication, US EPA, NHEERL, Atlantic Ecology Division, Narragansett, RI). It is not surprising that species such as *Fundulus heteroclitus* can adapt to chemical stresses, considering its short generation time and nonmigratory behavior. Except for studies with the tomcod and *Fundulus heteroclitus*, there are no other known reports of adaptation to AhR-active chemicals in other fish species. This is, however, a relatively new focus for toxicology, and it is likely that other 'resistant' species will be identified in the future.

3. Results of literature and data review: PCB congeners

Since the discovery of the structure activity relationship that exists between specific PCB congeners and toxicity (for those congeners that act via the AhR), there has been a great emphasis on determining the toxicity of specific PCB congeners. Until recently this emphasis has been on AhR-active PCB congeners, which include primarily the non-ortho substituted PCBs and the mono-ortho substituted PCBs. A summary of the laboratory and field studies reviewed and synthesized in this section are presented in Tables 8–10.

3.1. Laboratory studies: PCB congeners

The reproductive and developmental effects of specific PCB congeners in the laboratory are summarized in Table 8. The categories in this table are: species, con-

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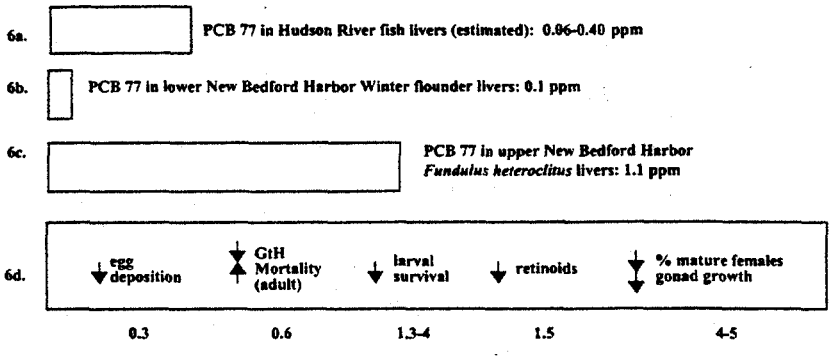

Fig. 6 (a-c). Comparison between PCB 77 concentrations estimated or measured in fish liver from the Hudson River (NY) (6a) and New Bedford Harbor (MA) (6b–c) [9,31,84], and concentrations of PCB 77 shown to cause reproductive and developmental effects in laboratory studies (6d).

gener, dose, tissue concentration (where concentrations are not reported, liver concentrations are estimated as described previously), effects, and references. Research on specific congeners is fairly recent in fish, and the majority of studies focus on the AhR-active congeners 77 and 126. Both congeners have a relatively high toxic equivalency factor (although PCB 126 is more toxic than 77) and are frequently present in environmental samples.

There were a sufficient number of studies on PCB 77 to construct a summary table and estimate effects resulting from various concentrations of PCB 77. Table 9 summarizes the data on PCB 77 presented in Table 8. Together these studies indicate that concentrations of PCB 77 ranging from 0.3 to 4.5 ppm in adult livers: reduce egg deposition, pituitary gonadotropin, and GSI; alter retinoid concentrations (vitamin A); and reduce larval survival. The study by Walker and Peterson [132] reports that 1.3 ppm injected into rainbow trout eggs reduces larval survival. A subsequent study by Walker et al., [136] found that LD50s (for dioxin) were similar for both egg injection and maternal routes of exposure, indicating that the egg injection technique is comparable to the more environmentally relevant maternal route of exposure. Three studies in Table 8 report reduced embryo and larval survival following exposure to concentrations of congener 126 ranging from 0.029 to 0.074 ppm (in the embryos or eggs), demonstrating the much greater potency of this congener compared to PCB 77.

Figure 6 provides a comparison of the effective concentrations for the reproductive and developmental effects of PCB 77 in fish and PCB 77 concentrations measured in fish from the Hudson River and New Bedford Harbor. The PCB 77 range for Hudson River fish fillets is from 0.03 to 0.2 ppm in fish fillets [84]. PCB 77 concentrations estimated in Hudson River fish livers (estimated to be twice the fillet concentration), range from 0.06 to 0.40 ppm (Fig. 6a). Mean PCB 77 concentrations in

Table 8
Reproductive and Developmental Effects of Laboratory Exposure to PCB Congeners

Species	PCB Congener	Dose (ppm)	Tissue Concentration (ppm)[a]	Effects	References
White perch	77	0.1, 1, 5.0 (i.p.) Three times over three months	0.4–0.8	none	[88]
			2.4–4.0	↓ % larval survival	
			3.7–4.5 (maternal liver)	33–58 % ↓ mature females, ↓ GSI (male and female), ~50 % ↓ larval survival.	
Fundulus heteroclitus	77	1, 10, 100 (i.p.)	0.3	48 % ↓ Egg deposition,	[7]
			3	77 % ↓ Egg deposition ↓ pituitary GT 25 % mortality	
			30 (estimated in liver)	95 % ↓ Egg deposition, ↓ pituitary gonadotropin, 50 % mortality	
Fundulus heteroclitus	77	0.2, 0.6, 2.0 (i.p.)	0.06	none	[7]
			0.18	28 % ↓ Egg deposition	
			0.6 (estimated in liver)	37% ↓ Egg deposition ↓ HSI, ↓ pituitary gonadotropin, 46 % mortality	
Striped bass	77	1 (i.p.) Three times over nine weeks	0.69–1.41 (in eggs)	No effect on estradiol No effect on testosterone No effect on vitellogenin	[87]
Brook trout (adult)	77	5 ppm (i.p.)	1.5 (estimated in liver)	↓ Growth rate, ↓ Plasma retinal, ↓ Intestinal retinoids, No effect on condition factor	[93]
Rainbow trout (juvenile)	77	5 (i.p.) assayed after 56 days	1.5 (estimated in liver)	↑ retinoic acid metabolism	[42]
Lake trout	126	0.6, 6.3, 25 i..p., sampled from 1 to 30 weeks post-dosing	No tissue estimate	↓ T_4 and T_3 after 3 weeks ↑ T_4 and T_3 at 6 and 13 weeks	[12]

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Table 8 (continued)
Reproductive and Developmental Effects of Laboratory Exposure to PCB Congeners

Species	PCB Congener	Dose (ppm)	Tissue Concentration (ppm)[a]	Effects	References
Lake trout	126	0, 0.003, 0.01 or 0.03 as single oral dose, sampled 12 weeks post-dosing	0.002	↓ dihydroretinol in liver and kidney, ↓ retinol palmitate and retinol in kidney	[100]
			0.007 and 0.02 (estimated in liver)	↓ dihydroretinol, retinol and retinol palmitate in liver and kidney	
Lake trout	126	Newly fertilized eggs exposed to 0.51–141 ng/L for 48 hr.	0.029 ug/g in eggs	LD50 (early life stage mortality)	[141]
Carp	126	Newly fertilized eggs exposed to 10^{-11}–10^{-9} mole/L for 48 hours, sampled 2–9 days after single exposure	No tissue estimate 10^{-11}	no effects on embryo or larval survival, ACTH or cortisol, ↑ α-MSH (pigment dispersing hormone)	[119]
			10^{-10} and 10^{-9}	↓ larval survival, ↑ ACTH, ↑ Cortisol, ↑ α-MSH	
Medaka	126	embryos exposed via water	0.046 ug/g	34 % embryo mortality	[69]
			0.093 ug/g in embryos, estimated from reported bioabsorption of approximately 31 %	61.6 % embryo mortality	
Lake trout	153	5 ppb via water (3X a day for 15 days, static) for 17 days	7.6 in sac fry	↓ % larval survival by ~89 %	[13]
Chinook salmon	153	5 ppb via water (3X a day for 15 days, static) for 17 days	3.6 in sac fry	↓ % larval survival by ~98 %	[13]
Medaka	126, 81, 77 individually	embryos exposed via water, doses ranging from 0.3–715 pmol/ml in water	126: 0.18 81: 2.34 77:>250 ng/ml in water, embryo concentrations are unavailable	LC50 for embryo mortality	[54]

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Table 8 (continued)
Reproductive and Developmental Effects of Laboratory Exposure to PCB Congeners

Species	PCB Congener	Dose (ppm)	Tissue Concentration (ppm)[a]	Effects	References
Rainbow trout	126, 77, 105, 118, 153 individually	embryos exposed via egg injection	126: 0.074 77: 1.3 105:>6.97 118:>6.97 153:>6.20	LD50 for early life stage mortality	[134]
fathead minnows	52, 101. 138, 153, 180 individually	prespawawning exposure in the water 2.5 ug/L or 25 ug/L	52: 9.8, 78.2 101:10.7, 78.6 138: 9.8, 69.2 153:10.4, 82.6 180:12.4, 80.9	No adverse effect on egg production, hatching or larval development	[121]
Rainbow trout	81, 169, 28, 118, 105, 156, 52, 170, 4, 128, 138 individually	embryos exposed via egg injection	81: 0.55 169: 7.11 28: > 24.3 118: > 57.4 105: >101.0 156: >115.0 52: > 30.4 170: > 41 4: > 24.2 128: >119.0 138: >130.0	LD50 for early life stage mortality	[142]

[a] Tissue concentrations, where reported, are in parts per million (ppm) wet weight. Where no concentrations are reported, they were estimated in the liver as described in the text. LD50 concentrations for egg injection studies are based on injected dose.

Table 9
Estimation of Effective Concentrations of PCB 77 in Fish from Laboratory Studies

Estimated or measured concentration of PCB 77 in liver (of adults), eggs or embryos (ppm)	Effects	References
0.3–0.5 (estimated adult liver)	↓ egg deposition	[7]
1.0 (estimated adult liver)	↓ reduced pituitary gonadotropin, ↑ mortality in adults	[7]
1.3 (estimated eggs)	↓ larval survival	[134]
2.4–4.0 (adult liver)	↓ larval survival	[88]
2.5 (estimated adult liver)	↑ retinoic acid metabolism, ↓ Growth rate, ↓ Plasma retinol, ↓ Intestinal retinoids, ↔ condition factor	[42,93]
3.7–4.5 (adult liver)	↓ % mature females, ↓ GSI (male and female)	[88]

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winter flounder from lower New Bedford Harbor and in *Fundulus heteroclitus* from the upper New Bedford Harbor are 0.1 and 1.1 ppm respectively (fig. 6b–c) [9,31]. Adverse effects on reproduction or development are summarized in Fig. 6d. Concentrations of PCB 77 in Hudson River and upper New Bedford Harbor fish are within range of PCB 77 concentrations shown to adversely affect reproduction in adult fish in laboratory studies. It is notable therefore that exposure to other PCB congeners that act similarly to PCB 77 (i.e., PCB 81, 126, 169) would increase the risk of these effects if the effects are AhR mediated and if toxicity is additive or synergistic.

3.2. Field studies: PCB congeners

Reproductive and developmental effects associated with PCB congener exposure in the field are presented in Table 10. Similar to laboratory studies, there are few field studies relating effects to specific congeners. Information presented in this table include: site, observation, contaminant concentration range associated with effects, species, and references. As with the PCB mixture studies, all of these sites are contaminated with other compounds.

To date, there are three studies that associate health effects to specific PCB congener exposure in the field. These studies report reduced embryo survival, reduced yolk sac survival and increased teratogenicity, as well as reduced intestinal retinoids (vitamin A, which may cause teratogenicity) in adult fish. All of these studies measure several different PCB congeners and acknowledge the presence of other contaminants at the field sites. It is therefore difficult to compare the findings from these field studies to the health effects caused by PCB 77 in the laboratory. There is some consistency between the finding of reduced retinoid concentrations following laboratory exposure to congener 77 and reduced retinoids measured in fish contam-

Table 10
Reproductive and Developmental Effects Associated with Exposure to Co-planar PCB Congeners in the Field

Site	Observation	Contaminants present at site	Concentration Range[a] ppm wet wt	Species	References
Riviere des Praries, Montreal	↑ Embryonic malformations	Co-planar PCBs Other chemicals	0.03 (E)	White sucker	[11]
Gulf of Bothnia	Yolk sac mortality (M74 syndrome)	77 126 169 and PCDFs Other chemicals	1 –77 0.27–17 0 – 2.4 These values are in ppm lipid weight	Baltic salmon	[133]
Saint Lawrence River	↓ Intestinal retinoids	77 and Co-planar PCBs Other chemicals	1.1 (E) 2.2 (E)	Lake sturgeon	[93]

[a] Concentrations associated with observation, concentration ranges or means are provided when no attempt was made to calculate an effect level. O = ovaries, L = liver, E = eggs.

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inated in the field with co-planar PCBs. In addition, as reflected in Table 9, the observation of reduced survival in offspring associated with exposure to co-planar PCB congeners is consistent with reduced survival of larvae exposed to these congeners in the laboratory [134] although the symptoms, when reported, are not the same. Walker and Peterson report the occurrence of blue-sac syndrome (yolk-sac edema and hemorrhaging) following laboratory exposure to PCBs, while Vourinen et al., [133] reported an association between the occurrence of M74 (sac fry mortality characterized by initial hyperactivity, exopthalmia, and brain lesions) and PCBs. It is notable that M74 and other early life-stage mortality syndromes were recently associated with thiamine deficiency, although the cause of this deficiency is currently unknown (reviewed in Marcquenski and Brown [78]).

3.3. Toxic equivalency quotients: PCB congeners

In order to provide a direct comparison of exposure to AhR-active PCBs and toxicity in Hudson River fish, TEQs are calculated for fish analyzed by McGroddy et al. [84]. The key to an accurate assessment using this approach relies on the toxic equivalency factors (TEFs) developed for each congener. Unfortunately, several different TEFs exist for each individual congener based on an array of *in vitro* and *in vivo* studies in fish. Partially in response to the problem of multiple TEFs, the European Center of Environmental Health of the World Health Organization and the International Program on Chemical Safety (ECEH-WHO and IPCS) met to assess and validate sets of consensus TEFs for mammals, birds and fish. The results of this meeting are summarized in Van den Berg et al. [129].

Consensus TEFs were defined as "an *order of magnitude* estimate of the toxicity of a compound relative to [dioxin]," developed by consensus after considering all relevant *in vivo* and *in vitro* data. The relative potency (REP) value is defined as the potency of a compound relative to dioxin as determined by a single *in vitro* or *in vivo* study. Clearly the methods used to derive the TEFs and REPs are critically important for the development and use of the TEF approach as a whole. Based on the ECEH-WHO/IPCS report, *in vivo* methods were given the most weight, followed by *in vitro* techniques, with derivations based solely on structure activity relationships given the least weight. In fish, there are two studies that derive REPs using early life stage mortality in rainbow trout [134,142]. The TEFs for fish recommended by the ECEH-WHO/IPCS are based mainly on these studies.

There are two main uncertainties associated with this approach. The first is the assumption that TEQs of individual congeners are additive (rather than antagonistic or synergistic). Several studies have addressed the issue of additivity and antagonism in fish [63,943,1375,141,1431]. A review of these studies and others concluded that "[i]t is unlikely that use of additivity in the TEF concept will result in a great deal of error in predicting the concentrations of TEQs due to synergism or antagonism" [1297]. Another uncertainty is the question of interspecies differences in the relative potencies or sensitivity to AhR-active compounds or the effects caused by

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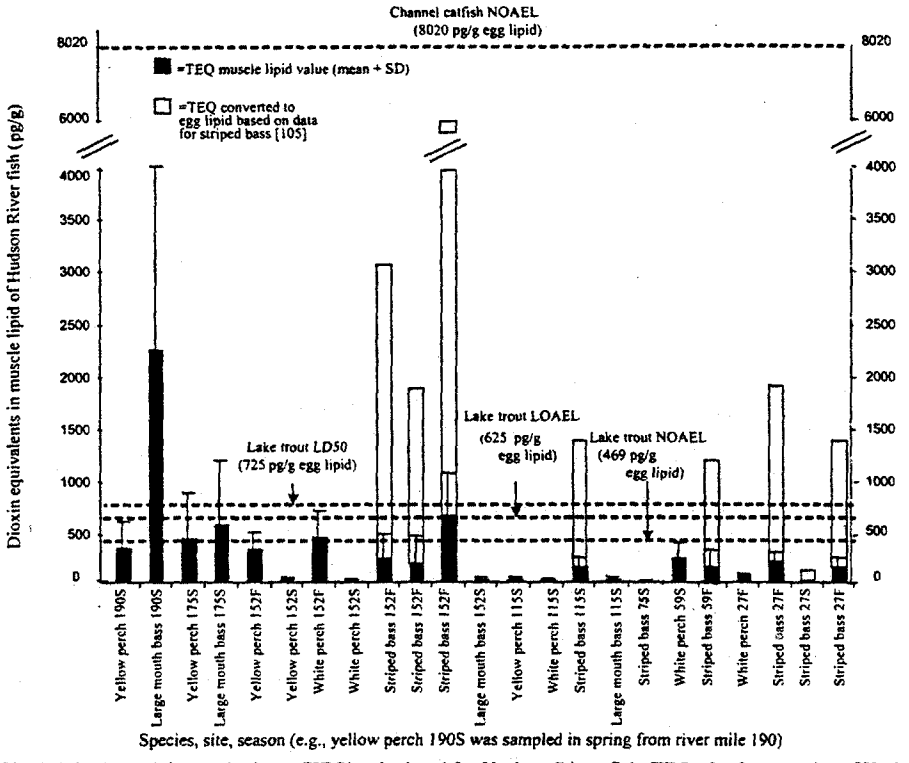

Fig. 7. Dioxin toxicity equivalents (TEQ) calculated for Hudson River fish. TEQs for four species of Hudson River fish were calculated for PCB congeners 77 and 126 measured in muscle tissue [84]. Values are for muscle lipid (closed bars). These TEQs are shown in comparison for the NOAEL reported for channel catfish [28] and the NOAEL and LOAEL for lake trout [137]. TEQs for striped bass were converted to egg lipid concentrations using data from Ray et al., [105] (these are approximate values since the conversion was for a population of striped bass Annapolis Bay and not the Hudson River) (open bars).

AhR-active compounds. This issue has also been addressed with regard to fish [28,135], and is discussed in detail in the section on interspecies differences (Section 4). Finally, it is important to note that this approach is useful for the assessment of AhR-active PCB congeners (and other AhR-active chemicals) only.

I use the TEFs suggested by the ECEH-WHO/ IPCS and chemistry data from McGroddy et al. [84] to calculate TEQs for four species of Hudson River fish collected from five sites along the river (Fig. 7). Based on occurrence and toxicity, the PCB congeners often considered of greatest importance in wild-caught fish are PCB 77, 81, 126, and 169 [19,54]. Detectable tissue concentrations of PCB 126, 77, and 81, but not 169 were reported in a limited study of Hudson River fish [58,59]. Thus, using the above criteria, the most important congeners in the Hudson River are PCBs 77, 81 and 126. Of these three congeners, McGroddy et al. [84] measured concentrations for PCB 77 and 126.[2] As a result, the TEQs for these fish are based on the concentrations

of congeners 77 and 126 only. The TEFs (for early life stage mortality in rainbow trout) for congener 77 and 126 are 0.0001 and 0.005 respectively [129].

TEQs for PCBs are calculated as follows: $TEQ = (\Sigma[PCB_i] \times TEF_i)$. For example, muscle tissue concentrations of PCBs 77 and 126 in a yellow perch caught at river mile 152 are 11.0 and 0.64 ng/g respectively [84]. Thus the $TEQ = (0.64 \times 0.005) + (11 \times 0.0001) = 0.0043$ ng/g. This TEQ of 0.0043 ng/g, or 4.3 pg/g (parts per trillion) means that this fish was exposed to the toxic equivalent of 4.3 pg/g of dioxin.

To provide some perspective on the TEQs for Hudson River fish, I compare these levels to dioxin LD50s (for early life stage mortality), lowest observed adverse effect levels (LOAEL) and no observed adverse effect levels (NOAEL)[3] for different fish species published in Walker et al. [134–136] and Elenon et al.[28]. The LD50s and LOAELs/NOAELs from these studies are based on PCB concentrations in either eggs (wet weight) or in egg lipid (not adult muscle tissue). Since PCB distribution is highly influenced by lipids, I convert the TEQs from wet weight muscle tissue concentrations to TEQs based on lipids in the muscle (the information necessary to convert muscle concentrations to egg lipid concentrations is not available for the McGroddy et al. [84] data-set). While this provides some degree of 'normalization,' there is still uncertainty with regard to comparisons across tissue type. The PCB distribution in the lipids of fish can vary with type of tissue, the type of lipid [72,120] and the relative lipid concentration between tissues [95].

The TEQs (muscle lipid) for Hudson River fish are shown in Fig. 7. In the figure, these TEQs are compared to the dioxin NOAELs for two other fish species, the channel catfish and the lake trout, and the LOAEL and LD50 (or $LC_{egg}50$) for lake trout. The data used for these comparisons are published in Elenon et al. [28] Walker et al. [134,136] and Guiney et al. [46]. These species were selected for comparison because lake trout are one of the most sensitive species to dioxin toxicity[4] (at this life stage) and channel catfish are moderately sensitive. The values for the channel catfish and the lake trout were converted from pg TCDD /g egg to pg TCDD/g lipid, using the percent of lipid reported in the eggs for each species (4.8 percent and 8 percent for channel catfish and lake trout, respectively).[5] Figure 7 depicts TEQs in Hudson River fish (bars) compared to lake trout LD50 (dashed line at 725 pg/g egg lipid), the lake trout LOAEL (dashed line at 625 pg/g egg lipid) and the NOAEL for lake trout and channel catfish (dashed lines at 469 and 8020 pg/g

2. Concentrations of PCB 126 were below detection limits for several fish. I estimated the PCB 126 concentration for these fish using one half the detection limit.

3. Authors refer to either LOAEL and NOAEL [46,134,136] or NOEL and LOEL [28], I use LOAEL and NOAEL in this paper.

4. A recent study indicates that the bull trout (Salvelinus confluentus), a species closely related to lake trout, is twice as sensitive to TCDD as lake trout [73]. However, the study did not include the NOAEL or LC50.

5. The data used to make these conversions are provided in Tables 2 and 5 in the Elonen et al.[28] study and in the text and Table 4 of Walker et al. [136].

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egg lipid, respectively) [28,46,134–136]. This figure indicates that the majority of muscle-lipid TEQs for Hudson River fish (which range from 3 to 2250 pg/g) are within an order of magnitude of the NOAEL, LOAEL and LD50 for lake trout, a sensitive species, but are one to two orders of magnitude below the NOAEL for channel catfish, a moderately sensitive species. This suggests that the cumulative toxicity values for PCB congeners 77 and 126 in Hudson River fish are likely below concentrations associated with early life stage mortality for species in families other than salmonidae.

As discussed earlier in this review, PCB distribution is influenced by tissue type in addition to lipid concentration. It would be most useful to compare TEQs based on egg-lipid concentrations. However, as noted above, egg-lipid data were unavailable. However, gonad-lipid concentrations from the muscle-lipid concentrations for striped bass could be estimated using data from Ray et al. [105]. Ray et al. [105] reported that the percent lipid in the gonads of striped bass from Annapolis Bay was 52 percent compared to 11 percent in muscle. Therefore, a factor of five is used to convert the TEQs based on muscle-lipid to TEQs based on gonad-lipid in striped bass.[6] The resulting estimated TEQs are shown in Fig. 7 as open bars. Compared to the very low muscle-lipid TEQs, the gonad-lipid TEQs for striped bass from river mile 152 to river mile 27 exceed the lake trout NOAEL and LOAEL. These estimated TEQs, however, are still far below the NOAEL for channel catfish. These results raise the question of interspecies sensitivity to dioxin, which will be discussed further in Section 4 of this review.

3.4. Discussion and conclusion: PCB congeners

There are few studies on the health effects of PCB congeners in fish aside from studies used to develop toxic equivalency factors. However, there is some consistency among the laboratory studies, in that larval survival is reduced by non-ortho (or AhR-active) PCB congeners. There are also data suggesting that a negative correlation exists between non-ortho PCB congeners and larval survival in the field. The concentrations of congener 77 shown to affect egg deposition in laboratory studies are within the range of congener 77 concentrations that may occur in some of the most highly contaminated Hudson River and New Bedford Harbor fish (Fig. 6). In addition there may be other non-ortho PCB congeners (e.g., 126 and 81) present in these fish that are also AhR-active, and are even more potent than congener 77 in causing embryo mortality [129,134].

One of the benefits of the TEQ approach, is that it is designed to account for exposure to mixtures of PCB congeners such as PCB 126 and 77 and other Ah-active contaminants. The TEQs for four species of Hudson River fish, as calculated in this review, are within an order of magnitude of the NOAEL, LOAEL and LD50 for di-

6. The authors reported that PCB concentrations were ten-fold higher in the gonad compared to the muscle although lipid concentrations were only five-fold higher in the gonad.

oxin in the most sensitive species known to date, the lake trout. These TEQs are more than an order-of-magnitude below the NOAEL for channel catfish, a moderately sensitive species. However, the TEQs were based on concentrations of congener 77 and 126 only and did not include PCB 81, which may also be present in Hudson River fish. In general, the conclusions drawn from the TEQ approach and estimation of effective concentrations of congener 77 are in agreement for Hudson River fish. In both cases the PCB tissue concentrations for the most contaminated Hudson River fish are within range of potentially toxic concentrations of PCBs. The calculated TEQs may not include all toxic PCB congeners present in Hudson River fish, however, and are therefore incomplete.

4. Interspecies differences: comparison of Hudson River fish species with laboratory and field test species

4.1. Background

One of the major uncertainties in this work is the potential for interspecies differences in response to PCBs. Differences in sensitivity to toxicants can be the result of an array of subtle variations in different biochemical and physiological systems, or the result of a single mutation affecting an organism's ability to detoxify certain chemicals (e.g., some differences in mammalian AhR may be the result of a mutation in a single gene or amino acid). Without a basic understanding of the relationship between these processes and environmental contaminants, it is not possible to accurately assess or predict the relative sensitivity across species. This is the case for the majority of contaminants and fish species. Differences in sensitivity may also be influenced by prior exposure to certain classes of environmental contaminants. While this is not very relevant to understanding interspecies differences in fish used for bioassays (e.g., acute and chronic toxicity tests), it is relevant to laboratory and field studies of native species as discussed earlier in this report (e.g., acquired resistance). Interspecies differences are also influenced by the specific chemical of concern. One chemical, for example, may cause different effects at different concentrations across species, another may cause similar effects at similar concentrations, or as demonstrated by dioxin [28], cause similar effects across species but at very different concentrations.

Ecotoxicological risk assessment often requires extrapolation across species. As a result, several different techniques have been developed to address this issue: uncertainty factors (based on NOAELs and LOAELs of acute toxicity data), allometric scaling (based on the premise that there is a linear relationship between size and acute toxicity), no extrapolation (with the assumption that similar species will behave similarly to different chemicals), and physiologically based pharmacokinetic modeling (PBPK), a data intensive process that utilizes metabolic and toxicity data that is directly relevant to the species of concern [16,56,107,115]. In general, there

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is no agreement on the most appropriate methodology for extrapolation, aside from the PBPK, for which the required data are usually not available. Of these methods, the use of uncertainty factors or the assumption of similar responses in similar species have been applied to fish [25].

Calabrese and Baldwin [16] suggest applying uncertainty factors that range from 10 to 65 for extrapolation across genus species to extrapolation across orders. These factors are based on binary comparisons of acute toxicity data across species for as many as 500 different chemicals and represent the mean uncertainty. It must be stressed that these factors are based on acute toxicity data, rather than chronic toxicity data. Although it is suggested that these uncertainty factors can be applied to chronic exposures [16], given the often more complex nature of chronic toxicity (e.g., absorption, distribution, and metabolism may all be more important in chronic events than in acute events) the appropriateness of using acute toxicity data for predicting chronic toxicity is currently unknown for the majority of chemicals in fish. It is also notable that in some cases the binary comparisons across orders such as Salmoniformes (used for many laboratory and field studies) vs Perciformes (which include four Hudson River species) can result in a response interval (used to calculate the uncertainty factor) that is more similar to the response interval associated with more closely related species (i.e., species belonging to the same family or genus). Thus, application of an uncertainty factor of 65 may result in overestimation of 'effective concentrations' if extrapolating across these two orders.

The assumption that similar species will behave similarly to chemicals may be valid in general for very closely related species. This premise was tested by the EPA when evaluating the use of surrogate species to assess contaminant risk to endangered species [25]. The results of that study suggest in general, that surrogate species respond similarly to acute chemical exposures, although for 30 percent of the comparisons, the surrogate or test species was less sensitive to toxicity compared to the endangered or listed species.

4.2. A1254, PCB mixtures and Hudson River fish

Since both of the methods discussed above involve consideration of genetic relatedness among species, Figs. 8 and 9 were developed to compare fish species used for either laboratory studies with A1254 and PCB mixtures in the field (Fig. 8), or species used for dioxin studies (Fig. 9) and "important" Hudson River species. Important is defined here as ecologically or commercially important, or endangered.

All of the fish species used for the laboratory studies cited in Table 2 (black typeface), field studies cited in Tables 7 and 10 (bold typeface), and the "important" Hudson River species (in grey typeface) are summarized in Fig. 8. The selection of Hudson River species was based on those used for chemical analysis by McGroddy et al. [84], and information provided by John Waldman of the Hudson River Foundation (personal communication). Where there was an overlap (i.e., a Hudson River species was used for either a laboratory or field study), the gray typeface is sur-

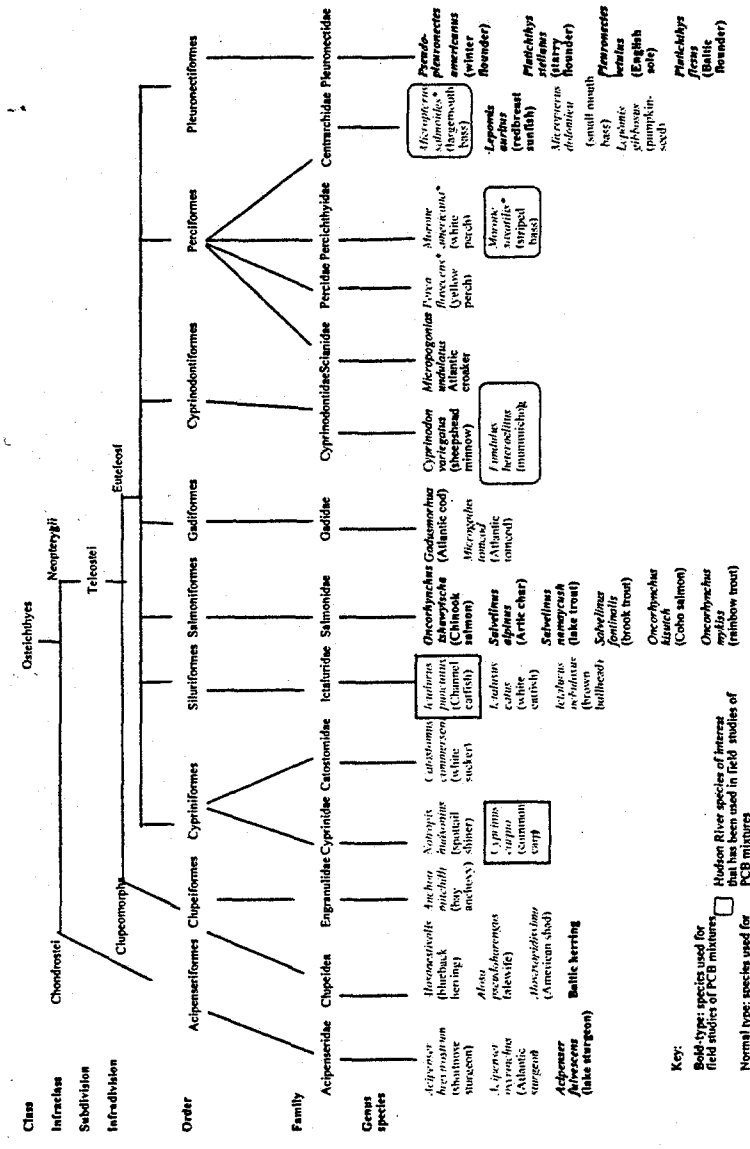

Fig. 8. Genetic hierarchy of species used for studies or reproductive and developmental effects of A1254 in the laboratory, PCB mixtures in the field, and Hudson River species of interest.

rounded by either a black box (for laboratory study) or a gray box (for a field study). An asterisk indicates that data on PCB concentrations are available for this species. For example largemouth bass are important to the Hudson, they were used in a field

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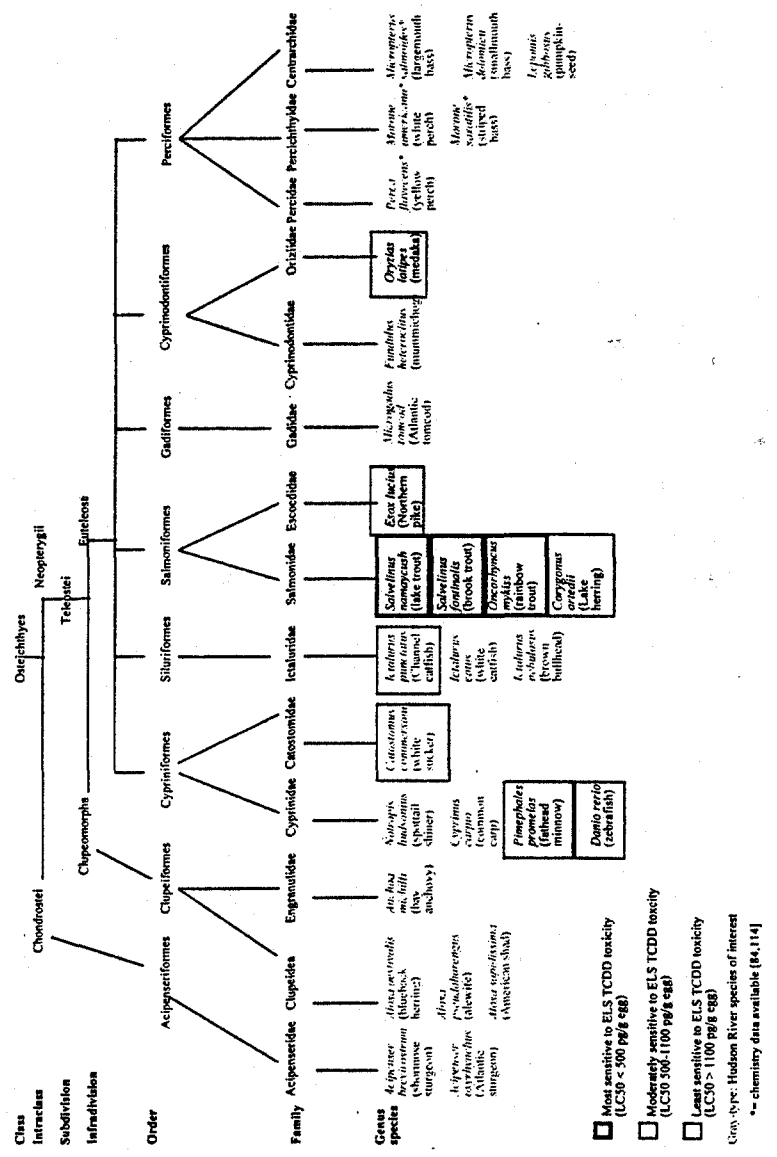

Fig. 9. Genetic hierarchy of species used for early life stage toxicity tests with dioxin and Hudson River species of interest. LC50 data for dioxin are from Elonen [28]; Walker et al., [136,137] and Walker and Peterson [134].

study of PCBs [39], and there are data on PCB concentrations in large mouth bass from the Hudson River [84]. In general, Fig. 8 shows there is little overlap between Hudson River species and those species used for laboratory or field studies.

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The relationships among all the species in Fig. 8 are shown using a hierarchical classification. This system is based on evolutionary relatedness however, unlike a true phylogenetic tree, this figure does not include geologic time. This system of classification provides a general overview of interspecies relatedness. As shown in Fig. 8, most species belong to the subdivision Teleostei, except for the sturgeons. The majority of species also belong to the infradivision Euteleosti, except for herring, shad, anchovy and alewife. Within Euteleosti, there are seven different orders, leading to 11 different families. In this figure, fish within the same genus are listed together (e.g., Salvelinus and Oncorhynchus are both listed directly under Salmonidae). Only two Hudson River species were used for laboratory studies of A1254 (common carp and channel catfish), and three Hudson River species have been used for field studies of PCBs (mummichog, striped bass and largemouth bass). However, several Hudson River species are closely related (within the same genus) to species used for either laboratory or field studies.

Based on the above discussion and the data presented in this report, it is not currently possible to estimate effective concentrations of PCBs for individual species residing in the Hudson River. However, the array of species shown in Fig. 8, many of which demonstrated reproductive or developmental toxicity following laboratory exposure to PCB concentrations that are (or are estimated to be) within an order of magnitude, include species belonging to six different orders of fish. In addition, species for which reproductive and developmental effects have been associated with PCB exposure in the field span two classes and six orders. These data suggest that PCBs cause reproductive and developmental effects to many different and distantly related species of fish, and that effective concentrations that begin at 25 ppm in adult liver and 5 ppm in larvae may apply to at least some species of Hudson River fish.

4.3. TEQs and Hudson River fish

Figures 9 and 10 provide some perspective on the range of sensitivity to dioxin, species relatedness, and species in the Hudson River. Similar to Fig. 8, relatedness among species is shown using a hierarchical classification. Species important to the Hudson River are shown in gray typeface, and species with known LC50s (based on early life stage mortality) for dioxin that are not Hudson River species are shown in black typeface. In Fig. 9, sensitivity to dioxin is demonstrated by boxes around the species, with graded line thickness, indicating high (LC50 < 500 pg/g egg), moderate (LC50 500–1100 pg/g egg) and low sensitivity (LC50 > 1100 pg/g egg). In Fig. 10, the LD50s for juvenile fish are listed below each species.

Figure 9 indicates little overlap between the species with known LC50s for dioxin and Hudson River species. The three most sensitive species all belong to the Salmonidae family. The remaining species for which there are LC50s are distributed among four orders and six families. These include two Hudson River species, white sucker (a least sensitive species) and channel catfish (a moderately sensitive species). There is no information on the Perciformes, which include several Hudson

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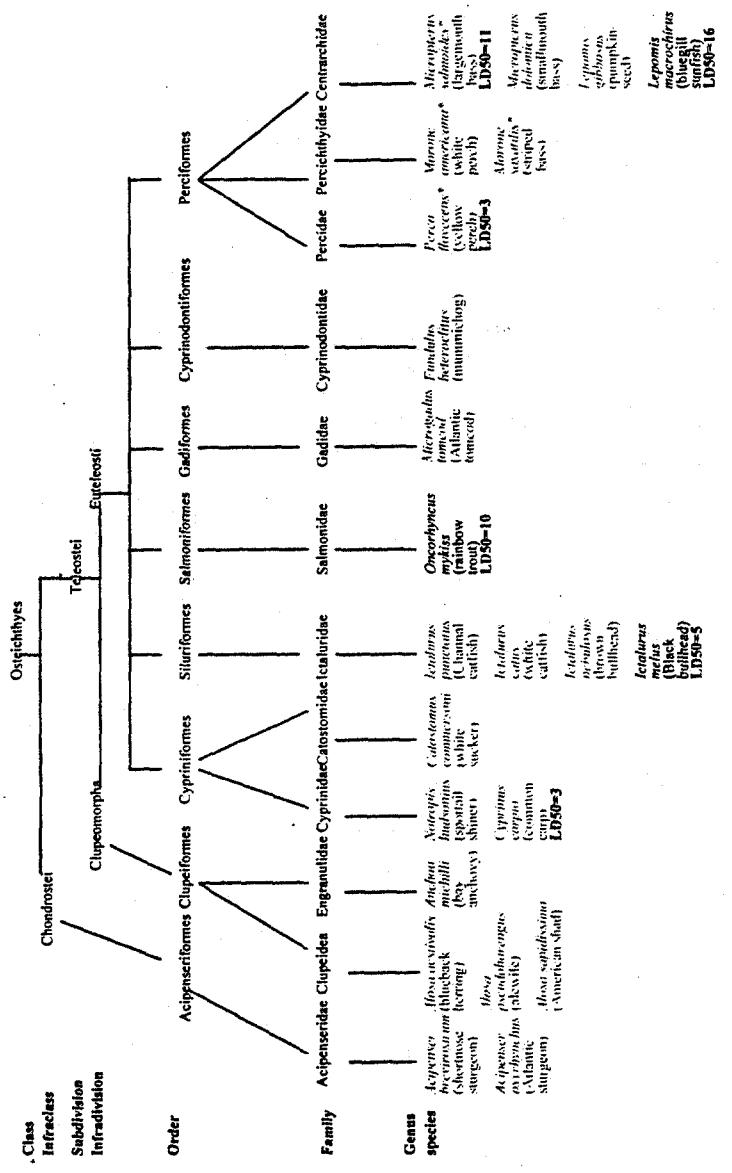

Fig. 10. Genetic hierarchy of species used for toxicity test with dioxin (at the juvenile stage) and Hudson River species of interest. LD50s for dioxin in juvenile fish are shown in bold [71].

River species, and the four species for which TEQs are calculated in this review. Figure 10 shows that the acute toxicity of dioxin to juvenile fish is known for three

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species of Hudson River fish (common carp, yellow perch and largemouth bass). It is interesting to note that the very large interspecies differences reported for early life stage mortality are not apparent at the juvenile stage for the species tested, with LD50s ranging from 3–16 ppb [71]. In addition, the juvenile fish are much less sensitive to dioxin toxicity than the larvae. Considering the large interspecies differences in early life stage toxicity, and the incomplete data set for PCB congeners, it is currently not possible to evaluate the risk to Hudson River fish larvae from exposure to co-planar PCBs using the TEQ method.

4.4. Conclusion: Interspecies differences

Consideration of interspecies differences, when synthesizing data is clearly of great importance, as shown by the interspecies differences in sensitivity to dioxin. However, data on a large array of species can be used advantageously. Many of the species included in this review demonstrated reproductive or developmental toxicity following laboratory exposure to PCB concentrations that are (or are estimated to be) within an order of magnitude. The array of species shown to respond to PCBs in the laboratory, and for which adverse effects associated with PCB exposure have been reported in the field, cover several phylogenetic orders. These data suggest that PCBs cause reproductive and developmental effects to many different and distantly related species of fish, and that effective concentrations that begin at 25 ppm in adult liver and 5 ppm in larvae may apply to at least some species of fish in the Hudson River and other contaminated sites.

5. Conclusion: Limitations and future approaches

'Effective concentrations' for both A1254 and PCB 77 were developed in an attempt to determine if PCBs affect reproduction and development at environmentally relevant concentrations. Although there were a relatively large number of laboratory and field studies available for this synthesis, I encountered a number of difficulties during the development of the effective concentrations. Three major difficulties in assimilating and using the existing data regarding the toxicity of PCBs were: 1) the variety of experimental designs used in laboratory studies (e.g. different routes and timing of exposure, dose regimen, fish species) 2) published laboratory studies did not always report tissue concentrations of PCBs and 3) the complexity of PCB mixtures (laboratory and field studies may use a variety of PCB mixtures, the composition of which can vary due to differential degradation, bioaccumulation and/or metabolism in the field or in the laboratory, thus both field and laboratory studies may report effects or concentrations of very different PCB mixtures).

Establishment of an effective concentration required the development of a common indicator of exposure, useful for synthesizing PCB exposure and effects data from a diversity of laboratory studies, that could be applied to both laboratory and

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field studies. I selected PCB concentrations in liver tissue for this purpose. PCBs accumulate in the liver and the liver is an important reproductive organ, producing vitellogenin in response to estradiol. However, PCB concentrations in fish tissue are not always reported in laboratory studies, and when concentrations are reported it is often in an array of tissues. When concentrations were not reported, liver PCB concentrations were estimated using available information including exposure dosages, exposure duration, routes of exposure, and extra-hepatic tissue concentrations. Clearly limitations exist in the use of this approach due to the lack of uniformity in the existing data base. The lack of a common basis for comparison of data generated by different laboratories proved to be the greatest impediment to understanding and synthesizing information on the reproductive effects of PCBs. Selection of a common 'unit' for inter-laboratory analysis by researchers in this field would be of great benefit to researchers and managers who may depend on these studies in the future. Analysis of hepatic PCB levels, for example, would generate the necessary data to validate and refine the conclusions drawn from the present assessment.

Another difficulty in assessing the toxicity of PCBs is the complexity of PCB mixtures. In the present assessment effective concentrations were established for both a PCB mixture (A1254) and for a specific PCB congener (PCB77). The advantage of the congener approach lies in the detailed understanding of the mechanism by which congeners that interact with the AhR elicit toxicity. Structure activity relationships have been established for these congeners. In addition there is a large, expanding database on the toxicity of these individual congeners in several different species of fish. This detailed understanding of AhR active PCB congeners (in addition to other AhR active chemicals) has led to the development and use of the TEQ approach for assessment of toxicity of PCB mixtures in wild-caught fish. One disadvantage of this approach is in its current limitation to AhR active compounds. Although it is clear that the more toxic PCBs are the AhR active congeners, non-AhR active congeners are present in much greater quantities in the field. Research elucidating potential mechanisms of reproductive and developmental toxicity of the non-AhR active PCBs is just beginning to emerge. In addition PCBs occur as complex mixtures in the field. While the TEQ approach provides a valuable tool for evaluating mixtures of AhR active PCBs, until more is known about all classes of PCBs and how they interact, it is still valid and important to consider PCB mixtures in addition to AhR-active congeners.

The effective concentrations for reproductive and developmental toxicity of PCBs in fish developed in this data synthesis incorporated as much of the currently available relevant information as possible. Clearly there were many uncertainties, in addition to those discussed above, that warrant further research. Studies that address interspecies differences in reproductive and developmental toxicity of PCBs, the toxicity of non AhR active PCBs alone or in the presence of AhR active PCBs, establishment of the validity of extrapolation from laboratory studies to animals exposed in the field, and the effects of additional chemicals (e.g. some metals and PAHs) present in combination with PCBs, would greatly reduce the number of un-

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certainties in this approach. As these uncertainties are addressed, the limitations associated with evaluating the reproductive and developmental effects of PCBs in fish will be reduced as well.

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