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Evaluation of Fish Early Life-Stage Toxicity Models of Chronic Embryonic Exposures to Complex Polycyclic Aromatic Hydrocarbon Mixtures

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Polycyclic aromatic hydrocarbons (PAHs) can cause a variety of effects in early life-stages of fish that have been chronically exposed as embryos, including mortality, deformities, and edemas. Mechanistic models of the chronic toxicity of complex mixtures of PAHs in fish have not been reported, with the exception of a previously untested model based on the lipids of fish as the site of action and toxicity caused through a narcosis mechanism. Four mechanism-based models of the chronic toxicity of embryonic exposures to complex mixtures of petrogenic PAHs in two species of fish, Pacific herring and pink salmon, were evaluated using a toxic-units approach: narcosis, aryl hydrocarbon receptor (AhR) agonism, alkyl phenanthrene toxicity, and combined toxicity. Alkyl phenanthrenes were the predominant PAH constituent determining early life-stage toxicity in both herring and salmon. The alkyl phenanthrene model had 67 to 80% accuracy in predicting the absence or presence of significant early life-stage toxicity, compared with a 40 to 50% accuracy and general underprediction of toxicity with the narcosis model. PAHs with high relative AhR affinity did not appear to contribute substantially to the observed early life-stage toxicity because of low concentrations of the most potent AhR agonists. Narcosis appeared to primarily contribute to embryo mortality and to be predominantly controlled by the concentration of naphthalenes. Except for the highest PAH exposure to herring, the primary toxic unit contribution to the combined toxicity model was alkyl phenanthrene toxicity to both herring and salmon. We recommend the continued use of total PAHs as a metric of exposure until mechanistic models have been further evaluated.

Key Words: **polycyclic aromatic hydrocarbon; aryl hydrocarbon receptor; narcosis; fish embryo.**

The acute toxicity of polycyclic aromatic hydrocarbons (PAHs) to fish is generally attributed to narcosis, and predictive models of acute toxicity have been available (Di Toro *et al.*, 2000; French-McCay, 2002). In aquatic organisms, narcosis is a reversible anesthetic effect that is caused by hydrophobic chemicals partitioning into cell membranes and nervous tissue that results in disruption of central nervous system function (Barron *et al.*, 2001). Chemicals causing narcosis in aquatic organisms are considered to have minimal toxicity, because critical body residues (CBRs) are substantially higher than CBRs for chemicals with more specific modes of action such as receptor-mediated toxicity (Barron *et al.*, 2001; McCarty and Mackay, 1993). Recently, Di Toro *et al.* (2000) have proposed a chronic toxicity model for PAHs based on the lipid of aquatic organisms as the site of action and toxicity caused through a narcosis mode of action. This model has not been tested for PAHs and Di Toro *et al.* (2000) cautioned that no experimental data have been presented to support the validity of the chronic tissue concentration determined for the narcosis-based target lipid model.

Recent research indicates that PAHs are unlikely to act as narcotic agents in early life-stages of fish that have been chronically exposed as embryos. Evidence includes sublethal effects that are not consistent with reversible narcotic toxicity and an absence of nervous system impairment that is characteristic of fish narcotic responses (Incardona *et al.*, in press). Sublethal effects caused by embryonic PAH exposures include edema of the yolk sac and pericardium, hemorrhaging, disruption of cardiac function, binding to the aryl hydrocarbon receptor (AhR) and CYP1A induction, mutations and heritable changes in progeny, craniofacial and spinal deformities, neuronal cell death, anemia, reduced growth, and impaired swimming (White *et al.*, 1999; Barron *et al.*, 2003; Billiard *et al.*, 1999, 2002; Brinkworth *et al.*, 2003; Marty *et al.*, 1997; Incardona *et al.*, in press). Many of these symptoms resemble the blue-sac disease caused by exposure to planar halogenated aromatic compounds (Brinkworth *et al.*, 2003; Hornung *et al.*, 1999; Incardona *et al.*, in press). In contrast, toxicity through a narcosis mechanism of action is characterized by central nervous system depression and rapidly reversible effects (Barron, 2002).

The objectives of this paper were to develop and evaluate mechanism-based models of the chronic toxicity of embryonic

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fish exposures to PAHs, and evaluate the models using independent data sets. The general approach was to first identify appropriate data sets that allowed dose-response assessments of chronic embryonic exposures to complex mixtures of PAHs and lethal and sublethal effects in fish early life-stages. The second step was to identify toxicity models based on presumed mechanisms of the embryonic toxicity of PAHs and to determine if any of the mechanistic models were better able to predict adverse effects in fish life stages. Four mechanism-based embryonic toxicity models were tested: narcosis, alkyl phenanthrene toxicity, AhR agonism, and combined toxicity. These mechanisms encompass the predominant theories on how PAHs cause toxicity in fish early life-stages: non-specific action of chemicals causing narcosis, receptor-mediated toxicity through interaction with the AhR receptor, and additive toxicity by multiple mechanisms. The mechanism of alkyl phenanthrene toxicity is unknown but may occur from oxidative stress and effects on cardiovascular morphogenesis (Brinkworth *et al.*, 2003; Incardona *et al.*, in press).

The purpose of evaluating embryonic toxicity models was to gain insight into the predominant mechanisms of PAH toxicity and evaluate the appropriate metrics of PAH exposure based on mechanism-determined differences in toxic potency. Two data sets were evaluated that consisted of embryonic exposures to complex mixtures of petrogenic PAHs in two species of fish (Carls *et al.*, 1999; Heintz *et al.*, 1999). Model results were also compared with a routine metric of exposure, total PAHs, which is the sum of detected parent and alkyl substituted PAHs and heterocyclic aromatics.

MATERIALS AND METHODS

Toxic unit approach. A toxic unit (TU) approach was used to evaluate four models based on narcosis, alkyl phenanthrene toxicity, AhR agonism, and combined toxicity. A total TU was calculated for each model from the sum of the fractional toxicity of each PAH in the mixture. The TU was computed from the PAH concentration in fish eggs that significantly increased early life-stage toxicity in one or more of the following endpoints during chronic embryonic exposures: mortality, deformities, edema, and growth and/or swimming performance. The potency of each PAH, as an egg concentration, differed in each model based on its presumed mechanism of toxicity. The TU approach was used because it allowed an assessment of both differences in the toxic potency of individual PAH compounds and the cumulative toxicity of bioaccumulated PAHs.

PAH exposure and toxicity data. PAH exposure and toxicity data were obtained from published data on chronic embryo exposures to petrogenic PAHs and resulting early life-stage toxicity in Pacific herring (Carls *et al.*, 1999) and pink salmon (Heintz *et al.*, 1999). These data sets were used to evaluate embryonic toxicity models because they provided high resolution chemical analyses of a complex mixture of 40 parent PAHs and their alkyl homologs during chronic embryonic exposures; methods are described in Short *et al.* (1996). Total PAHs were calculated from the detected parent PAHs and their C1-to-C4 alkyl homologs, including naphthalenes, acenaphthylene, acenaphthene, fluorenes, dibenzothiophenes, phenanthrenes, anthracene, fluoranthene, chrysenes, benzanthracene, benzofluoranthenes, perlyene, indeno[1,2,3-cd]pyrene, benzopyrenes, benzo[g,h,i]perylene, and dibenzo[a,h]anthracene. The Carls *et al.* (1999) and Heintz *et al.* (1999) data sets provided a comprehensive and rigorous evaluation of adverse effects on fish early life-stages, including the prevalence of mortality, deformities, edema, and reduced growth.

PAH concentrations in herring and salmon eggs generally increased to maximum tissue residues early in the exposure period, because water exposure concentrations declined over time as PAH reservoirs were depleted in the flow-through systems (Carls *et al.*, 1999; Heinz *et al.*, 1999). Aqueous exposure concentrations continuously declined because the more soluble PAHs partitioned into water that up-welled through oiled gravel columns that served as PAH reservoirs. Fish embryos were exposed to each PAH mixture only during the embryonic period, which varied by species. Herring eggs were exposed for the first 16 days of an approximately 30-day embryonic period. Pink salmon eggs were exposed for the entire embryonic period (~200 days). PAH concentrations in herring eggs at 4 days of exposure were selected for modeling, because maximum measured total PAH concentrations occurred at day-4 in four of the five oil exposures, and PAH concentrations were within 50% of the maximum in the fifth oil exposure. PAH concentrations in salmon eggs at 35 days of exposure were selected for modeling because maximum measured PAHs occurred at day 35 in all six oil exposures. Early life-stage effects of planar halogenated aromatic compounds are determined by early embryonic exposure (Hornung *et al.*, 1999) but may be less important for PAH toxicity (Brinkworth *et al.*, 2003).

Two attributes of the Carls *et al.* (1999) and Heintz *et al.* (1999) data sets allowed a quantitative assessment of multiple embryo toxicity models. First, multiple PAH treatments were tested in each species, which allowed an evaluation of early life-stage responses to increasing embryo exposures. Second, the complex mixtures of PAHs differed in PAH composition and thus differed in relative potency (Fig. 1), which allowed evaluation of early life-stage responses to treatments with different toxic potency. Egg concentrations were used to derive exposure concentrations because aqueous PAH concentrations were not constant during embryonic exposures, and tissue residues provided the most direct measure of embryonic exposure to PAHs. Tables 1 and 2 summarize the embryo treatments, PAH concentrations, and effects in the Carls *et al.* (1999) and Heintz *et al.* (1999) studies.

Embryo toxicity models. Embryo toxicity models were developed using literature data on tissue residues of PAHs associated with adverse effects that were independent of the data used in model assessment. Four toxic unit models were evaluated based on different mechanisms of PAH toxicity: narcosis, AhR agonism, alkyl phenanthrene toxicity, and combined toxicity. The accuracy of each model was assessed by determining the percentage of total correct model predictions of either no significant toxicity or significantly increased toxicity that occurred in each petrogenic PAH treatment (five herring and six salmon treatments). The TU approach predicts that toxicity will be significantly elevated at a TU of one. Significant observed effects that occurred between a predicted TU between 0.2 and 5 (within 5-fold of a TU of 1) were considered a correct prediction, and significant effects that occurred outside of this range were considered an incorrect prediction. A 5-fold range was selected to bracket a TU of one and allow for variability and uncertainty between measured and predicted responses. Significant toxicity was defined as a statistically significant ($p < 0.05$) increase in adverse effects for mortality, spinal deformities, and reduced growth in salmon and herring. A model was considered to underpredict toxicity if the calculated TU was below 0.2 in treatments with significant lethal or sublethal effects. Additionally, effects on yolk sac edema, pericardial edema, craniofacial malformations (reduced jaw size), and impaired swimming ability of herring were also assessed with the four embryo toxicity models. Tables 1 and 2 summarize the TU and PAH data used in embryo toxicity modeling.

Narcosis model. The narcosis model was based on the target lipid model of Di Toro *et al.* (2000) and their reported tissue-based final chronic value. Molar concentrations (μmol/g wet weight [ww]) of PAHs in herring and salmon eggs were computed from the measured concentration and molecular weight of each PAH; molecular weights for alkyl homolog groups were provided by Di Toro and McGrath (personal communication). Toxic units of narcotic compounds were determined from the ratio of the total molar concentration of PAHs in eggs and a lipid adjusted final chronic value for narcosis.

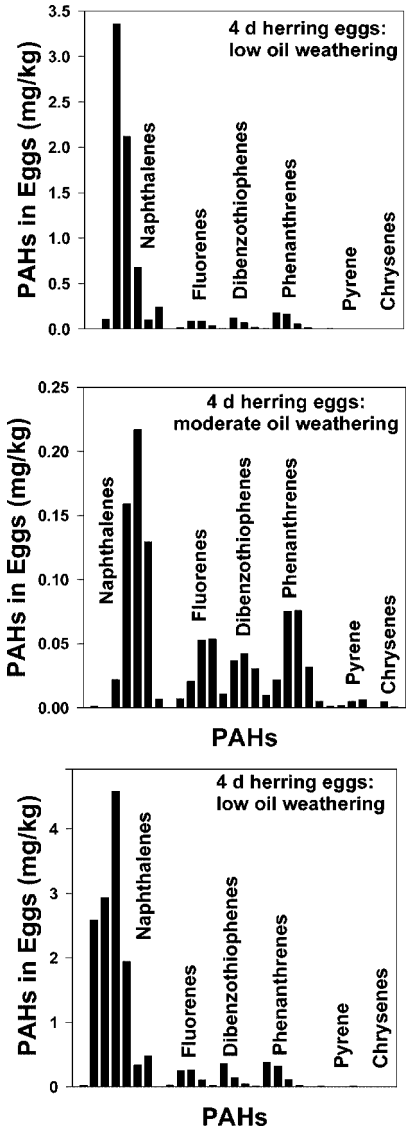

FIG. 1. Example PAH composition of herring eggs exposed to crude oil with either low weathering for four days (upper), moderate weathering for four days (middle), or low weathering for 16 days (lower).

The lipid adjusted final chronic value (μmol/g ww egg) was determined from the measured species-specific lipid content of Pacific herring and pink salmon eggs (Carls *et al.*, 1999; Heintz *et al.*, 1999). Specifically, the Di Toro *et al.* (2000) final chronic value for tissue concentrations of PAHs (3.79 μmol/g lipid) was converted to a herring egg concentration of 0.0516 μmol/g wet weight, using an average measured wet weight lipid content of 1.36% for Pacific herring eggs. The final chronic value was converted to a salmon egg concentration of 0.4813 μmol/g ww using an average measured wet weight lipid content of 12.7% for pink salmon eggs.

Alkyl phenanthrene model. The alkyl phenanthrene model was based on recent reports of high early life-stage toxicity in fish embryos exposed to alkyl phenanthrenes substituted with two to four carbons (C2 to C4 phenanthrenes). Brinkworth *et al.* (2003) reported early life-stage toxicity of rainbow trout exposed to 9 to 34 μg/l of the C4 alkyl phenanthrene (1-methyl-3-isopropyl phenanthrene; retene), including hemorrhages (yolk sac, pericardial sac, ocular and cranial tissues), yolk sac edema, skeletal deformities, and mortality. Tissue concentrations as low as 0.15 μg/g (0.00064 μmol/g) caused early life-stage toxicity in 100% of trout exposed as embryos (Brinkworth *et al.*, 2003). These results were also consistent with high early life-stage toxicity of retene, and substantially greater toxicity than parent phenanthrene observed by Hawkins *et al.* (2002). Kiparissis *et al.* (2001) reported that other alkyl phenanthrenes substituted with two to four carbons also had high early life-stage toxicity, with some two-carbon substituted phenanthrenes showing greater toxicity than retene. The alkyl phenanthrene model computed TU from the ratio of the total molar concentration of C2 to C4 phenanthrenes in eggs and a 0.00064 μmol/g toxicity value determined from Brinkworth *et al.* (2003).

AhR agonism model. The AhR agonism model was developed from previous findings that some PAHs can exhibit high affinity binding to the teleost AhR and early life-stage toxicity that resembled blue sac disease. The initial effort in model development was the derivation of fish potency factors (FPFs) relative to the AhR activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for over 50 PAHs (Barron *et al.*, in press). A FPF was assigned to each of the PAHs measured in herring and salmon embryos; the FPF was assumed to be 0 (no activity) for PAHs that were reported as inactive. The toxicity equivalence concentration (TEC; EPA, 2003) of PAHs in each egg was computed from the sum of the products of the concentration of each individual PAH and its corresponding FPF: TEC = 3[PAH]*FPF. The total TEC [TEC_{PAH}] was compared with the lowest observed effect concentrations for TCDD [TEC_{TCDD}] in eggs of two similar species: lake herring (LOEC: 0.270 ng/g; Elonen *et al.*, 1998) and rainbow trout (LOEC 0.279 ng/g; Walker *et al.*, 1992). The TU for each treatment was determined from: TU = TEC_{PAH}/TEC_{TCDD} (Tables 1 and 2).

Combined toxic units model. The combined toxic units model assumed that toxicity caused by narcosis, alkyl phenanthrene toxicity, and AhR agonism

TABLE 1
Total PAH Concentrations in Pacific Herring Eggs after 4 Days of Petrogenic PAH Exposure and Calculated Toxic Units (TU) for Embryo Toxicity Models

Embryo Treatment[a]	Total PAHs (mg/kg)	Narcosis TU	Alkyl-phenanthrene TU	AhR agonism TU	Combined toxicity TU	Significant effects[b]
Control LWO	0.015	0.002	0	<0.001	0.002	
Control MWO	0.015	0.002	0	<0.001	0.002	
Trace MWO	0.013	0.002	0	<0.001	0.002	
Low MWO	0.018	0.003	0.036	0.001	0.040	y, j
Mid MWO	0.227	0.021	0.566	0.008	0.600	y, d, g, p, j, s
High MWO	1.025	0.111	0.831	0.018	0.960	m, y, d, g, p, j, s
High LWO	7.445	0.941	0.523	0.026	1.489	m, y, d, g, p, j, s

[a]Treatments correspond to those reported in Carls *et al.* (1999). LWO, less weathered oil exposure; MWO, moderately weathered oil exposure.

[b]Statistically significant effects ($p < 0.05$) on embryo mortality (m), yolk sac edema (y), spinal deformities (d), growth (g), pericardial edema (p), jaw deformities (j), and swimming.

TABLE 2
Total PAH Concentrations in Pink Salmon Eggs after 35 Days of Petrogenic PAH Exposure and Calculated Toxic Units (TU) for Embryo Toxicity Models

Embryo treatment[a]	Total PAHs (mg/kg)	Narcosis TU	Alkyl-phenanthrene TU	AhR agonism TU	Combined toxicity TU	Significant effects[b]
Control	0.050	0.001	0.012	0.002	0.014	
Trace MWO	0.092	0.001	0.113	0.005	0.119	
Low VWO	0.142	0.002	0.235	0.0145	0.251	m, g
Low MWO	0.303	0.003	0.421	0.010	0.434	
Mid MWO	2.43	0.028	2.445	0.047	2.520	m, g
High MWO	5.74	0.068	2.207	0.063	2.339	m, g
Very high MWO	19.5	0.243	6.205	0.139	6.586	m, d, g

[a]Treatments reported in Heintz *et al.* (1999); qualitative descriptors added to be consistent with those used in Table 1. MWO, moderately weathered oil exposure; VWO, very weathered oil exposure.
[b]Statistically significant effects ($p < 0.05$) on embryo mortality (m), spinal deformities (d), and growth (g).

was additive. A total TU was computed from the sum of the TU determined for each mechanism-based model.

RESULTS

PAH Composition and Effects

PAH concentration and composition differed in both herring eggs (Fig. 1) and salmon eggs (Heintz *et al.*, 1999) exposed to weathered oil treatments. Eggs exposed to petrogenic PAHs derived from more weathered oil contained lower concentrations of naphthalenes and greater relative concentrations of PAHs with 3- and 4-fused rings (e.g., phenanthrenes). This was most apparent in salmon eggs exposed to the very weathered oil (VWO) treatment (Heintz *et al.*, 1999). Alkyl substituted PAHs were the predominant PAHs detected in fish embryos, which is characteristic of petrogenic PAH exposures (Fig. 1). Within each treatment, the PAH composition in eggs did not change appreciably over time (Fig. 1). Early life-stage toxicity was significantly elevated at total PAH concentrations of 0.018 to 19.5 mg/kg in herring and salmon eggs, where the total PAH concentration is the sum of 40 detected parent PAHs and their alkyl homologs (Fig. 2). Increasing total PAH exposure caused increasing early life-stage mortality, edema (yolk sac and pericardial), deformities (spinal and craniofacial), reduced growth, and swimming impairment (Fig. 2).

Narcosis Model

The narcosis model had 40% accuracy in predicting significant effects on herring in the TU range of 0.2 to 5 (within five-fold of a TU of 1), and generally underpredicted the degree of toxicity (Figs. 3 and 4). The narcosis model was able to predict egg mortality in herring (e.g., 56% mortality at a TU of 0.94), but the model was not a good predictor of sublethal effects (Figs. 3 and 4). For example, significantly ($p < 0.05$) elevated yolk-sac edema in herring occurred at narcotic TUs of 0.003 (26% incidence) and 0.021 (56% incidence) compared to a 5% incidence of edema in control fish (Fig. 3). Significant increases in pericardial edema (37%), spinal (23%) and craniofacial (44%) deformities, impaired swimming (28%), and reduced growth (19%) also occurred at a TU of 0.021; control fish exhibited less than 10% incidence of these effects (Figs. 3 and 4). Significantly increased mortality occurred at a TU of 0.1 in the narcosis model (Table 1).

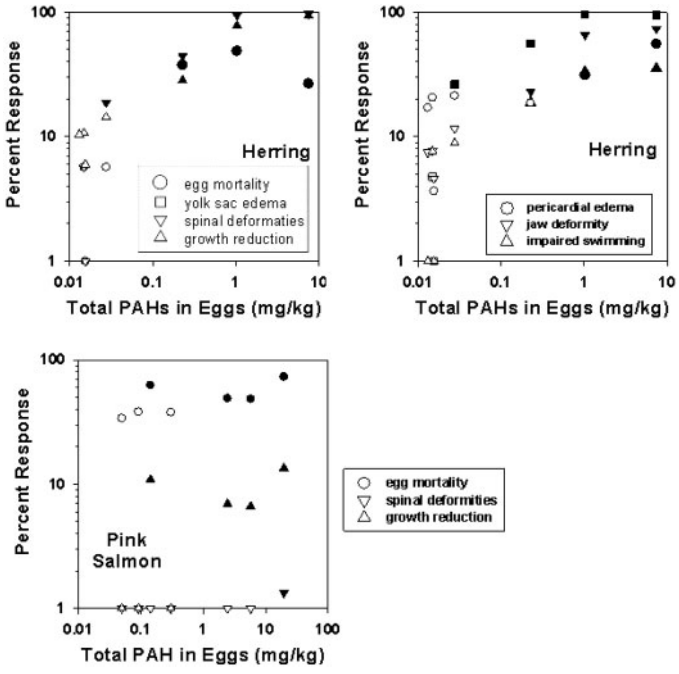

FIG. 2. Effects of embryonic exposures to complex mixtures of PAHs (mg/kg ww in eggs) on early life-stage responses of Pacific herring (upper panels) and pink salmon (lower panel): Symbols are defined within each panel; solid symbols indicate statistically significant differences from controls (Carls *et al.* 1999; Heintz *et al.* 1999).

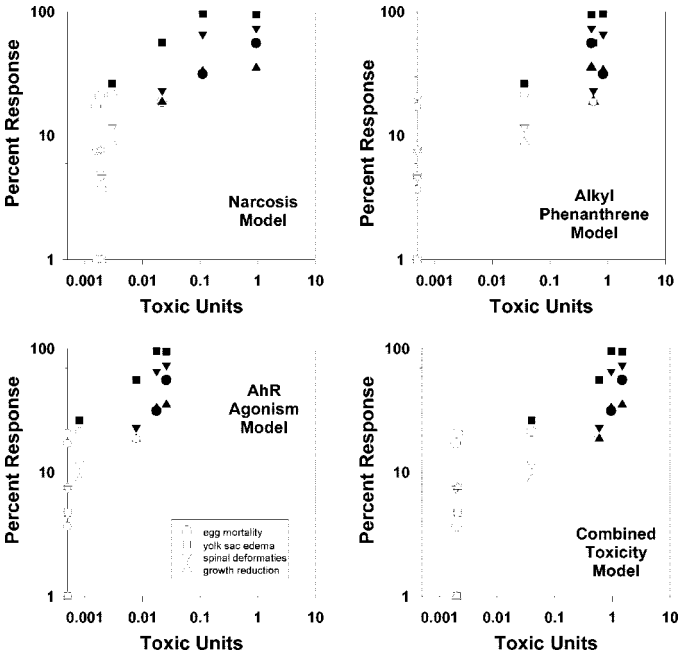

FIG. 3. Effects of embryonic exposures to complex mixtures of PAHs on Pacific herring early life-stage mortality, yolk sac edema, spinal deformities, and growth: Each panel shows the response versus toxic units for each of four mechanism-based models. Symbols for all four panels are defined in the AhR agonism panel. Solid symbols indicate statistically significant differences from controls (Carls *et al.* 1999; Heintz *et al.* 1999). Symbols that intersect the axes are off scale.

The narcosis model had 50% accuracy in predicting significant effects on salmon (0.2 to 5 TU), and generally underpredicted the degree of toxicity (Fig. 5). Only one of four PAH exposures with significant effects on mortality and growth were predicted to be toxic with the narcosis model (Table 2). Significantly increased mortality in MWO treatments occurred at a TU of 0.028 (Table 2).

Alkyl Phenanthrene Model

The alkyl phenanthrene model had 80% accuracy in predicting significant effects on herring (0.2 to 5 TU) and generally provided an accurate prediction of the degree of toxicity (Figs. 3 and 4). Significantly increased early life-stage toxicity occurred at TUs between 0.52 and 0.83, with significantly increased mortality at a TU of 0.8 (Table 1). The only underprediction of toxicity to herring occurred at a TU of 0.04 for yolk sac edema (26% incidence) and reduced jaw size (19% incidence).

The alkyl phenanthrene model had 67% accuracy in predicting significant effects on salmon (0.2 to 5 TU), and generally provided an accurate prediction of the degree of toxicity (Figs. 3 and 4). Significantly increased early life-stage toxicity (Table 2) occurred at TUs between 0.24 and 6.2, with significantly increased mortality in MWO treatments at a TU of 2.4 (Table 2). The model overestimated toxicity to salmon in the low MWO treatment (Table 2) with a TU of 0.42, whereas there were no significant effects on mortality, growth, or deformities.

AhR Agonism Model

The AhR agonism model underpredicted early life-stage toxicity for both herring and salmon (Figs. 3–5). Significantly increased mortality occurred at a TU of 0.02 in herring (Table 1), and a TU of 0.047 in MWO treatments in salmon (Table 2). Early life-stage responses of herring were highly correlated with TU (log-log r^2: 0.66 to 0.97 for the seven endpoints), but the maximum TU computed from the AhR agonism model was 0.026 (Table 1). Early life-stage responses of pink salmon had lower correlations with TU and a maximum TU of 0.14 (Table 2).

Combined Toxicity Model

The combined toxicity model had the same accuracy as the alkyl phenanthrene model, but had generally higher correlations between early life-stage toxicity and TU. Significantly increased mortality occurred at a TU of 1 in herring (Table 1), and a TU of 2.5 in MWO treatments in salmon (Table 2). With the exception of one herring treatment, the primary TU contribution to the combined toxicity model was alkyl phenanthrene toxicity to both herring and salmon (86–94% of total

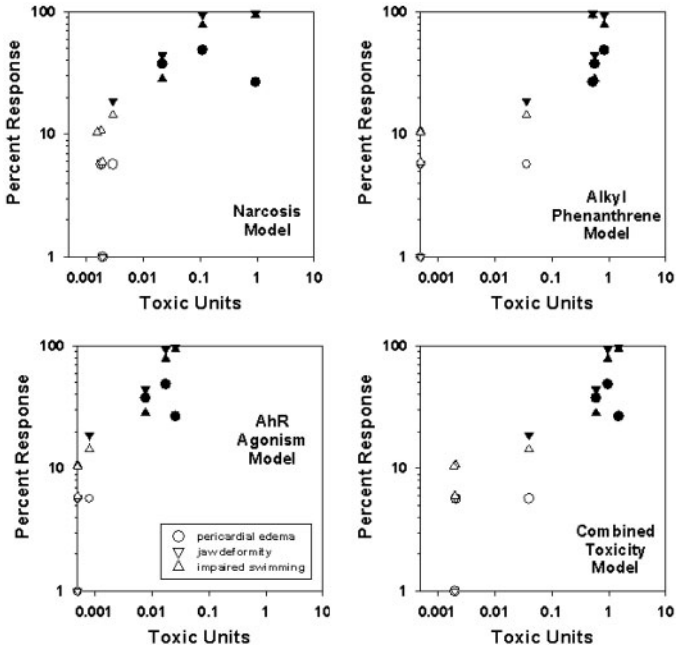

FIG. 4. Effects of embryonic exposures to complex mixtures of PAHs on Pacific herring early life-stage pericardial edema, craniofacial malformations, and swimming impairment: Each panel shows the response vs. toxic units for each of four mechanism-based models. Symbols for all four panels are defined in the AhR agonism panel. Solid symbols indicate statistically significant differences from controls (Carls *et al.* 1999; Heintz *et al.* 1999). Symbols that intersect the axes are off scale.

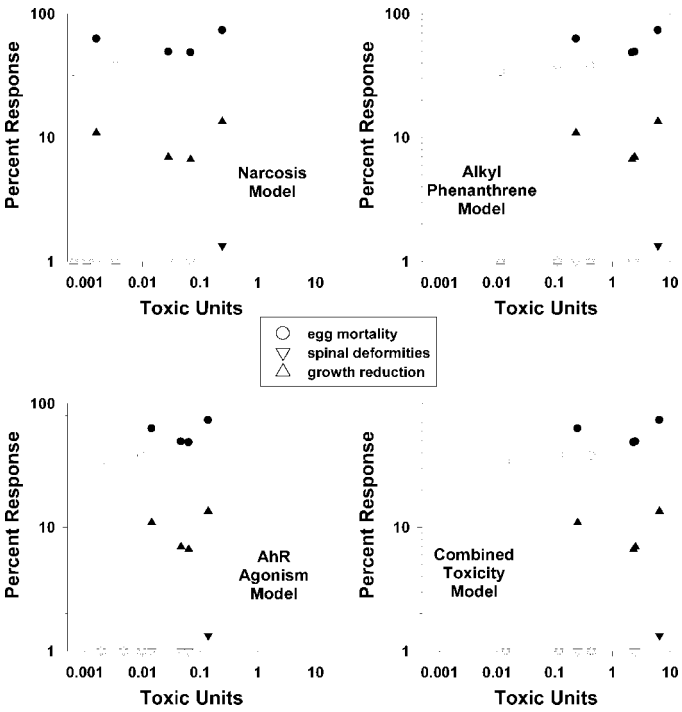

FIG. 5. Effects of embryonic exposures to complex mixtures of PAHs on pink salmon early life-stage mortality, spinal deformities, and growth: Each panel shows the response vs. toxic units for each of four mechanism-based models. Symbols for all four panels are defined in the center legend. Solid symbols indicate statistically significant differences from controls (Carls *et al.* 1999; Heintz *et al.* 1999). Symbols that intersect the axes are off scale.

TU). The only exception was the highest PAH exposure in herring, where narcosis contributed 63% of the total TU.

DISCUSSION

Recent research indicates that chronic PAH exposures in fish embryos can cause early life-stage mortality and a suite of sublethal effects that appear similar to the blue-sac disease attributed to more persistent planar halogenated aromatic compounds such as TCDD. Effects in a variety of fish species include early mortality, edema, hemorrhaging, disruption of cardiac function, and deformities (Barron *et al.*, 2003; Billiard *et al.*, 2002; Brinkworth *et al.*, 2003; Marty *et al.*, 1997; White *et al.*, 1999; Incardona *et al.*, in press). The embryonic toxicity of PAHs in fish appears to occur because of sensitivity to planar polycyclic aromatic compounds, high bioaccumulation and limited biotransformation, and exposure during critical developmental periods (Hornung *et al.*, 1999; Petersen and Kristensen, 1998; Incardona *et al.*, in press).

Mechanistic models of the chronic toxicity of PAHs in fish have not been reported, with the exception of the recently developed "target lipid" model that was based on a presumed mechanism of narcosis (Di Toro *et al.*, 2000). However the target lipid model was not specifically developed for the embryonic toxicity of PAHs and has not been tested. Four mechanism-based embryonic toxicity models were tested in the current investigation, including narcosis, alkyl phenanthrene toxicity, AhR agonism, and combined toxicity. These mechanisms encompass the predominant theories on how PAHs cause toxicity in fish early life-stages. A toxic unit approach was used because it allowed an assessment of both differences in the toxic potency of individual PAH compounds and the cumulative toxicity of bioaccumulated PAHs.

Adverse effects in Pacific herring and pink salmon early life-stages were interpreted relative to a TU of one. Specifically, statistically and toxicologically significant effects should increase as the TU approaches one, and more severe effects should occur above a TU of one. A model was considered to underpredict toxicity if calculated TUs were below 0.2 in treatments with significant lethal or sublethal effects. TU values were computed from egg residues early in the embryonic period because PAH exposures were near maximum levels during the critical developmental period for both species. With the exception of the high LWO herring treatment, all other TU values based on average concentrations during the exposure period would be lower, thus the majority of TU values determined in this study may be biased high by a factor of 2 to 10. However, time-weighted average concentrations could not be accurately computed because of limited sample data, including uncertainty in the time and concentration of highest egg exposures. The maximum measured concentrations used in computing TU may substantially underestimate actual maximum exposures based on disposition curves in Carls *et al.* (1999) and Heintz *et al.* (1999). Early life-stage effects of planar halogenated polycyclic aromatic compounds are determined by early embryonic exposure (Hornung *et al.*, 1999), but may be less important for PAH toxicity (Brinkworth *et al.*, 2003). Within each treatment, the PAH composition in eggs did not change appreciably over time, making this a more limited source of uncertainty and variability.

The alkyl phenanthrene model was able to generally predict both lethal and sublethal effects in herring and pink salmon from complex PAH mixtures, but the assumptions underlying the model require verification. Alkyl phenanthrene toxicity was based on a CBR for 1-methyl-3-isopropyl phenanthrene (retene) and observations that some two carbon substituted phenanthrenes exhibited greater toxicity than retene (Kiparissis *et al.*, 2001; Brinkworth *et al.*, 2003). The CBR was derived from the Brinkworth *et al.* (2003) study, where rainbow trout exposed as eggs for 0 to 7 days and those exposed until hatch (0 to 35 days) had similar residues (0.1 to 0.2 μg/g). The applicability of this CBR to the evaluation of alkyl phenanthrene mixtures in herring and salmon eggs is uncertain. In computing TU, all detected alkyl phenanthrenes with two to four carbons were summed, but research is needed to determine critical egg concentrations for a range of alkyl phenanthrenes.

The underlying mechanism for the alkyl phenanthrene

model has not been elucidated. The alkyl phenanthrene model was developed from the work of Hodson and coworkers (e.g., Kiparissis *et al.*, 2001; Hawkins *et al.*, 2002; Brinkworth *et al.*, 2003) evaluating the early life-stage toxicity of alkyl substituted phenanthrenes that are among the predominant PAH constituents of petroleum. Incardona *et al.* (in press) have shown that embryonic exposure to parent phenanthrene can cause direct effects on cardiac conduction that have subsequent secondary effects on cardiac morphogenesis, kidney development, neural tube structure and formation of the craniofacial skeleton. Alkyl phenanthrenes were not evaluated by Incardona *et al.* (in press), but Hawkins *et al.* (2002) demonstrated that retene (C4 phenanthrene) is over 100 times more toxic than parent phenanthrene. Hawkins *et al.* (2002) speculated that the abundance and persistence of metabolites mediates retene toxicity, but research is needed to elucidate the mechanism of the high early life-stage toxicity of alkyl phenanthrenes.

In contrast to the alkyl phenanthrene model, the narcosis model appeared to be mechanistically inconsistent with many of observed manifestations of early life-stage toxicity in PAH exposed embryos including edema, deformities, and cardiovascular dysfunction and failed to predict significant salmon embryo mortality and sublethal effects in both species. This result is not surprising given the derivation and presumed mode of narcotic toxicity in the Di Toro *et al.* (2000) model that was based on rapidly reversible effects associated with general narcotic depression (Barron, 2002). Di Toro *et al.* (2000) extrapolated acute toxicity data for a diversity of chemicals considered to cause narcosis in aquatic organisms (primarily solvents) to derive a final chronic toxicity value using an acute-to-chronic ratio based on more limited chronic toxicity data. PAHs had low representation in the data set used by Di Toro *et al.* (2000) to derive the target lipid model for PAHs (5%), and the few studies in the data set that incorporated embryo exposures appeared to exclude PAH exposure early in the embryonic period. The narcosis model likely has even lower accuracy than determined here because the CBR used to derive narcosis TU is biased low. Specifically, the CBR was a final chronic value (i.e., protective) rather than a tissue concentration associated with significant adverse effects. Use of a LOEC concentration would reduce the calculated TU proportionally; i.e., a 50% higher CBR results in a 50% lower TU and further underprediction of observed toxicity.

The AhR agonism model showed relatively high correlation between TU and early life-stage responses, but toxicity was substantially underpredicted in all cases. The inability of the AhR agonism model to accurately predict early life-stage toxicity may be explained by the general absence of the most potent high molecular weight PAHs such as benzo[*k*]fluoranthene and indeno[*1*,*2*,*3-cd*]pyrene (Barron *et al.*, 2004). Concentrations of PAHs in both salmon and herring eggs contained no PAHs larger than C3 chrysene, a four-ring PAH. The Barron *et al.* (2004) study indicated that common structural features associated with the highest relative AhR potency PAHs included four to six rings containing fluoranthene or phenanthrene structures with an exposed bay region. Two- and three-ring unsubstituted PAHs were consistently inactive in fish and avian and mammalian systems, and alkyl phenanthrenes had low relative AhR potency. However, relative potency was determined from AhR affinity or CYP1A1 induction, rather than embryonic toxicity in fish, which represents a substantial uncertainty in the AhR agonism model. Additional research is needed to determine whether relative potency based on AhR agonism is predictive of relative toxicity in fish early life-stages. Also, no potency data were available for application in the AhR agonism model for many of the alkyl substituted phenanthrenes and none of the dibenzothiophenes (Barron *et al.*, in press). This may result in an underestimation of TU, because these were two of the highest concentration constituents in herring and salmon eggs.

The combined toxicity model was based on the assumption of additive and concurrent toxicity for all three mechanisms of action. Alkyl-phenanthrene toxicity was the primary contributor to the combined-toxicity TU in both herring and salmon, but narcosis had a substantial TU contribution at high-PAH exposures in herring. The results of this model evaluation confirm the importance of alkyl-phenanthrene toxicity in petroleum toxicity that has been noted by Hodson and coworkers (e.g., Brinkworth *et al.*, 2003; Hawkins *et al.*, 2002), and also suggests that the toxicity of complex mixtures of PAHs may occur through multiple mechanisms of action. This is consistent with the conclusions of Incardona *et al.* (in press) that embryonic exposures of fish to different PAH compounds have distinct and specific effects on fish early life-stages. PAHs with the highest relative AhR affinity (Barron *et al.*, in press) did not appear to contribute substantially to the observed early life-stage toxicity in herring and salmon because of low concentrations of the most potent AhR agonists. Narcosis appeared to primarily contribute to embryo mortality and be predominantly controlled by the concentration of naphthalenes. Each of the mechanistic models assumed additivity, which is consistent with narcosis (Di Toro *et al.*, 2000) and AhR agonism (EPA, 2003), but is unknown for alkyl-phenanthrene toxicity. One surprising finding was that no model was able to predict the toxicity of the VWO exposure in pink salmon, which contained higher proportions of more persistent high-molecular-weight PAHs, but which had relatively low apparent TCDD-like potency (Barron *et al.*, in press). Additional research is needed to evaluate whether the AhR agonism and combined toxicity models will be predictive of pyrogenic PAH exposures that have higher proportions of the most potent AhR agonists.

Finally, despite its simplicity, the expression of total PAHs as a metric of complex-mixture exposures has merit. Adverse effects in herring and pink salmon were correlated with total PAH concentrations despite substantial differences in PAH composition and likely potency differences. Although not a mechanistic model, the total PAH metric implicitly assumes

additivity and similar potency in complex mixtures of PAHs. We recommend the continued use of total PAHs as a metric of exposure until mechanistic models have been further evaluated.

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