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Analyses of Laboratory and Field Studies of Reproductive Toxicity in Birds Exposed to Dioxin-like Compounds for Use in Ecological Risk Assessment

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National Center for Environmental Assessment
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NOTICE

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LIST OF ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
CASRN	Chemical Abstract Service Registry Number
ED_x	Effective dose for x (percent of test subjects)
ERA	Ecological risk assessment
EROD	Ethoxyresorufin-O-deethylase
FEL	Frank effect level
GLM	General linear model
HC_x	Hazardous concentration for x (percent of tested species)
LD_x	Lethal dose for x (percent of test subjects)
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
PBT	Persistent bioaccumulative toxicant
PCB	Polychlorinated biphenyl
PCDD/F	Polychlorinated dibenzodioxin/furan
PHAH	Polyhalogenated aromatic hydrocarbon
REP/TEF	Relative potency/Toxicological equivalency factor
RTECS	Registry of Toxic Effects of Chemical Substances
SAS	Statistical analysis system
SSD	Species sensitivity distribution
TCDD	Tetrachlorodibenzo-p-dioxin
TEC	Toxicological equivalent concentration
TEQ	Toxicity equivalent concentration
TRV	Toxicity reference value

EXECUTIVE SUMMARY

This report is intended to assist ecological risk assessors who must characterize risks to birds from exposure to dioxin-like chemicals. Those chemicals include the halogenated dibenzo-dioxins, dibenzo-furans, and biphenyls that have the same mode of action as 2,3,7,8-tetracholorodibenzo-*p*-dioxin. In particular, they include the coplanar PCBs, which account for most of the toxicity of PCB mixtures. They have been shown to severely affect birds in contaminated sites and regions by causing mortality, deformity and inhibited development of embryos and hatchlings.

Effects of dioxin-like chemicals in the field may be assessed in multiple ways. The most accurate way is to perform tests of the mixture that occurs in the field. For example, one may collect contaminated fish from the contaminated site and feed them to birds or extract the contaminants and inject them into eggs. However, that approach is costly and time consuming. An alternative, where PCBs are the contaminants of concern, is to use toxicity data for the commercial PCB mixtures. However, the PCBs found in food items in the field are quite different from the original commercial mixtures. The last approach, presented here, is to measure or estimate the concentrations of individual congeners and relate them to appropriate toxicity data. This approach is made possible by the ability to convert the toxicity of all dioxin-like chemicals to common toxic equivalent concentrations (TEQ) and then adding the TEQ values to estimate the exposure to the mixture as an equivalent concentration of 2,3,7,8-tetracholorodibenzo-*p*-dioxin. This approach has its own uncertainties, but it has the advantage of allowing assessment of diverse dioxin-like chemical mixtures without testing.

The exposure metric used in this report is µg/kg of egg as TEQ. The laboratory data are based on egg injections and the field data are based on measured egg concentrations. Most of the laboratory data are for domestic chickens, but ten other species of birds have also been tested. Chickens are the most sensitive avian species

tested, but their sensitivity does not appear to be aberrant relative to other sensitive species.

Multiple approaches are considered for estimating risks to a particular bird species or community. Common methods include using the most sensitive species to represent all species, using a similar species, or using the most sensitive species with an uncertainty factor. These approaches use only one effects datum, so the other available information is lost. The species sensitivity distribution (SSD) approach uses the distribution of effects concentrations for all species. Hence, just as conventional dose-response curves can be used to estimate the probability of effects for an individual human, the SSD can be used to estimate the probability of effects on a species. However, for these chemicals, effects levels for the most sensitive species are approximately equal to the 5-10% levels of the SSD which are commonly used as benchmark values. Hence, the methods are concordant for dioxin-like effects on birds.

The TEQ concentrations in eggs in the field that induced death or developmental defects were generally lower than the corresponding laboratory values. The effects levels for chickens and the low end of the laboratory SSDs correspond to effects on 25-50% of species in the field. The difference is believed to be due to effects of non-dioxin-like co-contaminants in the field. However, other factors such as parental behavior may also be involved.

Since death or developmental defects in embryos or hatchlings are the critical effects of dioxin-like chemicals in birds, the results presented in this report are believed to be useful for screening assessments. The screening benchmark for an assessment may be chosen from values presented here based on the assessment endpoints and the preferences of the assessors and risk managers. Use of these values for more definitive assessments must be based on the expertise of an assessor who is knowledgeable concerning the effects of these chemicals on birds. When practical, tests of site-specific mixtures should be conducted to provide a more accurate characterization of risk.

1. INTRODUCTION AND GOALS

This report is intended to summarize in a useful manner exposure-response information for birds from laboratory and field studies of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally and mechanistically related (dioxin-like) compounds. The data are derived from two prior reports, including their recent updates (U.S. EPA, 2001b, 2002). Those reports contain the results of literature searches that included a range of aquatic and terrestrial organisms and diverse modes of exposure and types of effects. The analyses and interpretations presented here are limited to a subset of the data presented in those reports. As explained below, the focus on reproductive effects on birds is intended to meet an important need of ecological risk assessors and to take advantage of the fact that those effects have been reported in a relatively consistent manner that lends itself to quantitative analysis.

Dioxin-like compounds are those that are believed to have the same mechanism of action as TCDD. They include the PCDDs and PCDFs substituted in at least the 2,3,7,8 positions and the structurally and toxicologically similar non- or mono-*ortho*-substituted tetra-, penta-, hexa- and hepta- chlorobiphenyl congeners (PCBs), and their bromine-substituted analogues. There are 135 PCDD congeners, 75 PCDF congeners, and 209 PCB congeners theoretically possible. The common mechanism is referred to as aryl hydrocarbon receptor- (AhR-) mediated toxicity. AhR-mediated effects result from PCDDs, PCDFs and PCBs binding to the AhR in the cytosol, which then binds to a translocating protein that carries this activated TCDD-AhR complex into the nucleus. In the nucleus, the binding of these activated complexes to specific DNA sequences results in gene transcription alterations, including the induction of cytochrome P4501A enzyme (CYP1A). Taxa exhibiting AhR-mediated effects include mammals, birds and fish. Further description of the role of this mechanism in ecological effects may be found in U.S. EPA (2001a).

The goal of this report is to provide to ecological risk assessors a relatively consistent set of avian toxicity data for dioxin-like chemicals and a useful set of alternative analyses of those data. Each of those alternatives may be useful in a particular assessment context. It is important to recognize that none of the values or relationships presented here constitute in any sense a criterion, standard, TRV, or other U.S. EPA-endorsed benchmark. Rather, the appropriateness of any estimated threshold value or effects level must be determined by the risk manager or other decision maker in consultation with risk assessors. Similarly, although we believe that the type of data used here are, in general, the most appropriate for estimating risks to birds from dioxin-like chemicals, other data may be more appropriate in specific cases. The full literature reviews are found in U.S. EPA (2001b, 2002).

2. APPROACH

This section describes the approach taken to this report and explains the intent and rationale for that approach. Specific methods for deriving exposure-response relationships are discussed in the following section.

2.1. DATA SOURCES

The data used in this report were obtained from previously published literature searches (U.S. EPA, 2001b, 2002). The search terms included common names, chemical name synonyms and registration numbers such as CASRN (Chemical Abstract Service Registry Number) for each congener. A second list of search terms included potentially affected wildlife species (fish, birds, mammals, reptiles/amphibians and invertebrates). A third list contained an extensive array of ecotoxicological endpoints. Electronic searches were conducted for studies published in peer-refereed journals which contained one or more terms from each list. Papers were retained if they contained all of the following:

- More than one quantitative dose or exposure. The many single exposure studies were not included because of the uncertainty of their interpretation in a dose-response context.
- One or more quantifiable, toxicological endpoint was identified
- Appropriate statistical tests showing significant changes in response as dose or exposure levels change
- The study authors evaluated the potential of co-contaminants to bias the results in the field-exposure studies

For the selected studies, information on the experimental design or field study design, exposure, and effects was recorded and entered into an electronic data base. The searches included toxicological information from laboratory studies of the full set of taxa and from field studies with birds. The searches extended back to 1980 and were last updated in mid-2002. A subset of those laboratory and field data sets was used in this study. They were studies of avian embryo or hatchling mortality, deformities, or

other developmental effects which were accompanied by concentrations in eggs. That data set is presented in Appendix A. The criteria for selection of data for these analyses from the prior literature reviews are presented in Box 2-1.

TEXT BOX 2-1. Criteria for data selection

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The studies used in these analyses were selected from those in the literature reviews in U.S. EPA (2001b, 2002). The criteria used for selecting studies to for analysis were based on the criteria described in Appendix D to Part 132 Great Lakes Water Quality Initiative (GLWQI) Methodology for the Development of Wildlife Criteria (U.S. EPA, 1993, 1995). Those criteria were refined as follows to produce sufficiently consistent data sets.

Included:

- Studies of any avian species
- Laboratory studies in which exposure was by egg injection
- Laboratory studies that expressed exposure as egg concentrations of individual dioxin-like chemicals or defined mixtures of dioxin-like chemicals
- Field studies in which exposure was expressed as or could be converted to egg concentrations in TEQs
- Laboratory studies in which the reported effects included mortality or developmental decrements or defects of embryos or hatchlings
- Field studies in which the recorded effects included mortality, developmental decrements or defects of embryos or hatchlings, or reductions in fledging success
- The NOEC and LOEC for the most sensitive appropriate response

Excluded:

- Effects on enzyme induction or other effects that are not considered adverse
- Studies in which exposure was defined as concentrations of an Aroclor or other commercial mixture
- Laboratory studies in which chicken eggs were injected after day four or the equivalent developmental stage for other species

2.2. USE OF INDIVIDUAL CONGENERS

This report assesses the individual compounds and estimates their toxicity in a common unit, mg/kg egg 2,3,7,8-TCDD equivalents (TEQs). There are three alternatives to this approach. First, one may perform tests of the actual mixture of concern collected at the contaminated site (Summer et al., 1996; Halbrook et al., 1999). This is the most reliable approach, but it is expensive and time consuming. Even if such tests are a potential option, some screening assessment method is required to

determine where they are justified. The second approach is to use published toxicity information for whole PCB product mixtures such as the seven Aroclors marketed in the U.S. or equivalent products marketed elsewhere (Chapman, 2003). This approach is not appropriate if halogenated dioxins or furans are present in significant amounts. Even if PCBs are the only contaminants of concern, this approach is questionable. In the years since PCB use was halted, component PCB congeners have undergone differential degradation and partitioning so that the mixtures in abiotic media differ from the original mixtures. In addition, differential uptake by biota, which occurs at each step in a food chain, results in dietary exposure to a mixture that differs from that in the abiotic media. These weathered and bioaccumulated mixtures tend to be more toxic than the parent product mixture (Giesy and Kannan, 1998). Further, toxicity data for specific Aroclors or other product mixtures are often unavailable for taxa of interest. The last alternative approach is to use total PCBs as the exposure concentration which may be related to effects data for some PCB mixture. This reduces the problem of data availability and the fact that ambient concentrations cannot be accurately represented as Araclor concentrations. However, total PCB exposure concentrations cannot be matched to toxicity data for any particular tested material. One solution is to use data from a study in which weathered and bioaccumulated PCBs in biota from a site are used to expose test organisms (Giesy and Kannan, 1998). One such study, in which contaminated carp were fed to chickens, is available in Summer et al. (1996). That approach requires that the site mixture be sufficiently similar to the tested mixture. Since there is no guidance on how to judge that the similarity is sufficient, the judgement must be ad hoc (U.S. EPA, 2000a).

The use of individual compounds to assess risks from dioxin-like toxicity has advantages and disadvantages. The chief advantage is that it provides flexibility in addressing a wide variety of mixtures. High-resolution analytical techniques now allow the characterization and quantification of individual congeners in abiotic or biotic materials. While avian toxicity data are not available for all dioxin-like compounds, the

development of Toxicity Equivalency Factors (TEFs) allows estimation of the effects of the individual members of the group or of the combined toxicity of the dioxin-like constituents of contaminant mixtures. One significant limitation of this approach is the uncertainty associated with the TEFs. They are described as order-of-magnitude estimates (van den Berg et al., 1998). A second disadvantage of this approach is that effects that are not mediated by the Ah receptor are not included. Some congeners that weakly bind the Ah receptor may be more toxic through other mechanisms of action, and the *ortho*-substituted PCBs that do not bind to the Ah receptor are not represented in this method. Because non-dioxin-like mechanisms are not well known, there is no good way to address them currently other than testing the ambient mixture. Hence, the TEF approach used in this report estimates risks arising from only one mechanism of action. One may assume that the dioxin-like effects are the only ones that need be considered when assessing halogenated dioxins, furans and PCBs. This assumption is supported by the fact that, even for PCB mixtures, the AhR-mediated effects are the critical effects in tests on animals (Giesy and Kannan, 1998). Critical effects are the biologically significant effects that occur at the lowest exposures and would result in the lowest allowable total concentration in environmental media. Alternatively, one may simply assume that this approach addresses one important mechanism of action for halogenated dioxins, furans and PCBs, and other mechanisms must be addressed separately. More research is needed concerning those other mechanisms of action of halogenated dicyclic aromatic compounds. A final disadvantage is the cost of analytical chemistry for the many compounds in contaminated media.

The use of TEFs to toxicity-normalize the concentrations of dioxin-like compounds and to estimate their combined toxicity in mixtures is based on their concentration-additivity (U.S. EPA, 2000a). Chemicals with a common mechanism of action have parallel concentration-response curves, so concentrations of one may be converted to effective concentrations of another by multiplying by a factor. If one chemical's toxicity is well-characterized, the concentrations of the other members of the

group may be converted to equivalent concentrations of that chemical by multiplying by the appropriate TEFs. The product of the concentration of a chemical and its TEF is the toxicity equivalent concentration (TEQ). The effective concentration of a mixture of such chemicals may be estimated by adding the converted concentrations to derive a TEQ for the mixture (TEQ_m). That is,

$$TEQ_m = \sum(TEF_i * c_i) \quad (1)$$

where, c_i is the concentration of an individual compound and TEF_i is the corresponding factor. In this case, the well-characterized index chemical is 2,3,7,8-TCDD and the TEQs are estimates of mixture concentrations equal to the same concentration of that dioxin. The TEFs for birds (Table 2-1) were estimated by a WHO expert panel based on all available scientific data (van den Berg et al., 1998). A U.S. EPA report suggested that the TEF approach and the WHO values for the calculation of risks from coplanar PCBs and PCDD/Fs to fish and wildlife are useful for ecological risk assesment, and they are used by U.S. EPA asessors (U.S. EPA, 2001a; Valoppi et al., 1999). However, ecological risk assessments based on Aroclor concentrations are still found to be useful in some U.S. EPA regions (Chapman, 2003).

Although the use of congener concentrations and TEFs to estimate risks has conceptual difficulties and quantitative uncertainties, it has proven to be useful in practice. TEQs are well correlated with effects on avian populations in the field and normalization using TEFs reduces variance in toxic exposure levels among studies (Giesy et al., 1994; Giesy and Kannan, 1998).

TABLE 2-1

Chemical Compounds with Known or Probable Ability to Cause Aryl Hydrocarbon Receptor-mediated Toxicity to Fish and Wildlife. WHO Consensus TEFs for birds from van den Berg et al. (1998)

Chemical Compound	Abbreviation	TEF
Polychlorinated dibenzo-*p*-dioxins (PCDDs)		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	TCDD	1.0
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	1,2,3,7,8-PeCDD	1.0
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	1,2,3,4,7,8-HxCDD	0.05
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	1,2,3,6,7,8-HxCDD	0.01
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	1,2,3,4,6,7,8-HpCDD	<0.001
Polychlorinated dibenzofurans (PCDFs)		
2,3,7,8-Tetrachlorodibenzofuran	TCDF	1.0
1,2,3,7,8-Pentachlorodibenzofuran	1,2,3,7,8-PeCDF	0.1
2,3,4,7,8-Pentachlorodibenzofuran	2,3,4,7,8-PeCDF	1.0
1,2,3,4,7,8-Hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF	0.001
1,2,3,4,7,8,9-Heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF	0.001
Non-*ortho* chlorinated polychlorinated biphenyls (co-planar PCBs)		
3,4,4',5-Tetrachlorobiphenyl	PCB 81*	0.1
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	0.05
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	0.1
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169	0.001
Mono-o*rtho* chlorinated polychlorinated biphenyls		
2',3,4,4',5-Pentachlorobiphenyl	PCB 123	0.00001
2,3',4,4',5-Pentachlorobiphenyl	PCB 118	0.00001
2,3,4,4',5-Pentachlorobiphenyl	PCB 114	0.0001
2,3,3',4,4'-Pentachlorobiphenyl	PCB 105	0.0001
2,3',4,4',5,5'-Hexachlorobiphenyl	PCB 167	0.00001
2,3,3',4,4',5-Hexachlorobiphenyl	PCB 156	0.0001
2,3,3'4,4',5'-Hexachlorobiphenyl	PCB 157	0.0001
2,3,3',4,4',5,5'-Heptachlorobiphenyl	PCB 189	0.00001
Polybrominated analogs of PCDDs, PCCDFs, PCBs and PCDEs (analogs of above compounds)		

*IUPAC PCB numbering system

2.3 BIRDS AS ENDPOINT ORGANISMS

Although other classes of organisms were included in the literature searches and summaries of laboratory data (U.S. EPA, 2001b), the review of field studies and this report are limited to analysis of effects on birds. Birds were selected because they are known to be sensitive to PCBs and dioxin-like compounds. In addition, birds are top predators in many systems, so they are highly exposed to these biomagnified compounds. Finally, effects of dioxin-like compounds on birds have been a concern at specific contaminated sites such as the Fox River, Wisconsin, and regionally in the Laurentian Great Lakes and elsewhere.

All appropriate data of adequate quality for birds were included, but the inclusion of the domestic chicken (*Gallus domesticus*) has been questioned. Data for chickens were retained, because there was no reason to expect that domestication has made them inherently more or less sensitive to toxic chemicals. Although they are sensitive to dioxin-like compounds, they are insensitive to some other chemicals such as some cholinesterase-inhibiting pesticides (Smith, 1987). The fact that chickens are the most sensitive tested species for dioxin-like compounds, might suggest that they are somehow inherently different from wild birds with respect to that mechanism of action. However, the sensitivity is not considered aberrant for two reasons. First, sensitivities to dioxin-like chemicals are extremely variable for all vertebrate taxa. Hence, the fact that chickens are a more than a factor of 100 more sensitive than other avian species in some test sets is consistent with the large differences in sensitivity between guinea pigs and other mammals. Second, the gap between chickens and other birds may be a function of the relatively small number of avian species tested. In terms of NOAELs (the most abundant test endpoint, with ten species tested), chickens are on average only a factor of 2.5 more sensitive than the next most sensitive bird, the American kestrel (*Falco sparverius*) (Table 2-2). Hence, chickens are sensitive relative to other tested birds, but evidence does not suggest that there is not an inherent mechanistic difference between chickens and other

TABLE 2-2

Geometric Means of NOAELS, LOAELS, and LC_{50} Values for Developmental Impairment from Laboratory Studies of Birds Exposed to Dioxin-like Compounds (TEQs as µg/kg of Egg)

Species[a]	NOAEL	n[b]	Prop.[c]	LOAEL	n[b]	Prop.[c]	LC_{50}	n[b]	Prop.[c]
Gallus domesticus	0.066	28	0.05	0.15	30	0.08	0.16	5	0.1
Falco sparverius	0.23	1	0.15	3.39	2	0.25	10.13	2	0.7
Phasianus colchicus	0.71	2	0.25	7.94	3	0.58	1.72	2	0.3
Phalacrocorax auritus	3.67	4	0.35	11.09	4	0.92	8.41	2	0.5
Meleagris gallopavo	10.00	1	0.45	10.00	2	0.75			
Anas platyrhynchos	35.36	2	0.55						
Anser anser	50.00	1	0.80						
Bucephala clangula	50.00	1	0.80						
Larus ridibundus	50.00	1	0.80						
Larus argentatus	50.00	1	0.80						
Sterna hirundo				4.40	1	0.42	10.40	1	0.9

[a] For common names, see Appendix B

[b] Number of tests

[c] Proportion of ranked species

birds that would preclude the possibility that some species of wild birds are equally or more sensitive.

Chickens are not recommended by the U.S. EPA for avian pesticide testing, but not because their sensitivity is unusual. Rather, they are not appropriate for reproduction tests because of their high egg production, and acute tests are not performed on species that are not used in reproduction tests (Edward Fite, U.S. EPA Office of Pesticide Programs, personal communication). Hence, the reason for not using chickens in pesticide testing does not apply to these tests and field studies, because egg production is not an endpoint.

2.4. MEASURES OF EFFECTS

Dioxin-like chemicals have a variety of effects including enzyme induction, immunotoxic effects and cancer. However, this report addresses effects on the survival and development of avian embryos and chicks. These effects were chosen because of data availability, comparability among studies and the clear relevance of reproductive success to avian populations. Embryo developmental and lethal effects constitute the most common test endpoints for effects of dioxin-like chemicals on birds, because they appear to be the most important sensitive effects for those chemicals (Giesy and Kannan, 1998). Further, embryo lethality, based on in ovo exposures, is the preferred response for the derivation of avian TEFs (van den Berg et al., 1998). This is also consistent with the proposed soil screening levels for wildlife, which use reproductive data preferentially for all chemicals (U.S. EPA, 2000b). Two types of effects endpoints are analyzed. First, an aggregate endpoint including lethality to embryos (failure to hatch) or to hatchlings, deformities, and reduced growth was used. These effects were considered to be effectively equivalent because deformed and poorly developed birds are less likely to survive and reproduce. In addition, mortality, deformity, growth retardation, and edema co-occur in birds exposed to dioxin-like chemicals, so that they may be considered a syndrom rather than discrete effects (Gilbertson et al., 1991). Hence, the deformities and lethalities will be referred to here as developmental effects,

because failure to hatch or survive after hatching represents the extremity of developmental failure. This aggregate developmental endpoint is needed to compare the laboratory data to the field data, which are less consistent and more focused on deformities. Second, for the sake of consistency, the mortality data from laboratory tests were analyzed, without the deformities or growth effects.

From each study, one or more of the following measurement endpoints for reproductive and developmental effects were obtained from the study:

- NOAEL - No-Observed-Adverse-Effect Level. This is the highest egg concentration from a study that did not have a statistically significant effect on mortality or development.
- LOAEL - Lowest-Observed-Adverse-Effect Level. This is the lowest egg concentration from a study that had a statistically significant effect on mortality or development.
- LC_{50} - Median Lethal Concentration.
- FEL - Frank-Effects Level, defined here as an exposure level causing high mortality, up to total reproductive failure, of a nesting colony.

All effective concentrations were converted to consistent units, µg TEQ/kg egg, wet weight.

2.5. EXPOSURE METRICS

The exposure metric is the concentration in eggs, expressed as 2,3,7,8-TCDD toxicity equivalents (TEQs), wet weight. Egg concentrations were used because they are the most directly relevant exposure metric for effects on development, and because they can be compared among laboratory and field studies. In addition, the use of egg concentrations should reduce the interspecies variance by avoiding the variance among species in uptake and toxicokinetics as well as the variance among oral toxicity tests due to variance in the administered form. Concentrations may result from egg injections or from maternal contribution. These modes of egg contamination appear to be equivalent in their effect on the developing chick, if injections occur early in development. After preliminary analysis, data from studies that injected eggs after day four in chickens or at a comparable stages of development in other species were

eliminated to obtain a data set based on effectively equivalent exposures. After day four, chicken embryos have developed all organs and are less susceptible to developmental toxicity.

Egg concentrations may be used in two ways in ecological risk assessments. First, eggs may be collected at a site and the measured concentrations, normalized to TEQs, may be related to the effects information presented here. Second, the concentrations in eggs may be estimated by modeling from concentrations in abiotic media or in prey organisms (U.S. EPA, 1993; MacIntosh et al., 1994). The estimated TEQ concentrations may then be compared to the effects concentrations presented here.

2.6. LABORATORY VERSUS FIELD STUDIES

Avian effects data are available from both laboratory toxicity tests and field studies of birds at contaminated sites. Each type of study has advantages and disadvantages. Laboratory studies allow control of exposure, replication, and random assignment of treatments. Hence, the differences among exposure groups and controls can be assumed to be caused by the treatment or error. However, laboratory studies are always subject to the criticism that conditions or the mode of exposure are unrealistic. Field studies are inherently realistic, but are inevitably uncontrolled, unrandomized and, at best, imperfectly replicated. Hence, field studies are subject to confounding. The most obvious confounding factor is the presence of contaminants other than dioxin-like compounds. Other differences between field sites may confound results by affecting the size and quality of the eggs, the nest-attentiveness of the adults, or genetic characteristics of the populations. In addition, the treatment levels used for estimating field NOAELs, LOAELs and FELs are imprecise. They are based on binning the continuum of egg concentrations in intervals and then choosing a concentration to represent each interval. Hence, the laboratory and field results represent alternative estimates of the effects of exposure to dioxin-like compounds, each with strengths and weaknesses.

2.7. ALTERNATIVE EXTRAPOLATION MODELS

Currently, there are no standard models for estimating effects on one wildlife species or a wildlife community from data concerning a set of test species. Hence, we take the approach in this report of applying multiple methods to the problem of estimating risks to birds.

3. METHODS AND RESULTS

Ecological risk assessors must determine how to use existing data for multiple species to estimate the effects on individual avian species or the avian community. This section considers the utility of the common approaches to that problem for risks from dioxin-like chemical effects. It does not include techniques such as toxicokinetic modeling which are beyond the current state of practice, particularly for embryonic exposures.

3.1. USE THE SPECIES OF CONCERN

One solution to the extrapolation problem is to avoid it by using data from the species of concern (i.e., an assessment endpoint species). A relatively large number of avian species have been tested or studied in the field for their responses to dioxin-like compounds (U.S. EPA, 2001b, 2002). If one of them is present at a contaminated site and is sufficiently significant, it might be selected as an endpoint species. Alternatively, new tests or field studies may be performed on a species that has been selected for its significance at a site. However, there are some constraints on new studies. Some bird species are difficult to obtain, to maintain or to breed in the laboratory. Field studies have been largely limited to colonial-nesting birds, because of the difficulty of defining treatment groups and observing enough eggs and hatchlings with solitary-nesting species. Hence, using data for the endpoint species is a good option that is not likely to be available for most assessments.

3.2. MOST SENSITIVE TESTED SPECIES

It is common practice in risk assessment to use the most sensitive tested species to represent all endpoint species. This approach is assumed to be conservative. However, if few species are tested, it is likely that some species will be more sensitive than the most sensitive tested species. For example, if five species are tested, the most sensitive species represents the lower 20^{th} percentile of species. Even if we assume that the most sensitive species is exactly the 10^{th} percentile species (i.e., it is at the

midpoint of its range), in a 100 species avian community, ten would be expected to be more sensitive. Given the sigmoid shape of most species sensitivity distributions, some of those species may be considerably more sensitive.

Chickens are the most sensitive avian species tested with dioxin-like chemicals (Table 3-1). As discussed above, there is no objective reason to not use data for chickens, and in fact they have been used to derive TRVs (Chapman, 2003).

3.3. MOST SIMILAR SPECIES

Rather than choosing the most sensitive tested species, it may be advisable to choose the most similar tested species. Similarity of toxic response is correlated with taxonomic similarity in a variety of taxa (Suter, 1993). In addition, taxonomic patterns of sensitivity have been important in practice. For example, the observed levels of DDT/E in peregrine falcons or bald eagles did not appear to be sufficient to account for reproductive effects, until testing was done on a member of the same order (Lincer, 1975). This generalization appears to be borne out by the data for dioxin-like developmental effects (Tables 2-2 and 3-1). Based on laboratory NOAELs (the test endpoint available for the most species), the three galliform birds are all more sensitive than average, and the three anseriform birds cluster at the median or lower. Using this approach, one might, for example, choose the kestrel test results for an assessment of risks to osprey (*Pandion haliaetus*), because they are both members of the Falconiformes. Since there are field data for osprey (Table 3-2), we can check the result and see that the kestrel laboratory value (0.23 µg/kg TEQ) is within a factor of two of the osprey field value (0.14 µg/kg TEQ). Similarly, the LOAEL for Common tern (*Sterna hirundo*) in the laboratory (4.40 µg/kg TEQ) is close to the Caspian tern (*S. caspia*) in the field (1.42 µg/kg TEQ). These examples do not validate the approach, but they serve to illustrate its potential utility. As a counter example, the wood duck

TABLE 3-1

Geometric Means of NOAELs, LOAELs for Embryo Mortality and LC_{50}s from Laboratory Studies of Birds Exposed to Dioxin-like Compounds (TEQs as µg/kg of Egg)

Species[a]	NOAEL	n[b]	Prop.[c]	LOAEL	n[b]	Prop.[c]	LC_{50}	n[b]	Prop.[c]
Gallus domesticus	0.068	18	0.056	0.21	21	0.083	0.16	1	0.1
Phasianus colchicus	0.71	2	0.17	7.94	3	0.58	1.72	1	0.3
Phalacrocorax auritus	3.67	4	0.28	11.09	4	0.92	8.41	1	0.5
Meleagris gallopavo	10.00	1	0.39	10.00	2	0.75			
Anas platyrhynchos	35.35	2	0.50						
Anser anser	50.00	1	0.78						
Bucephala clangula	50.00	1	0.78						
Larus argentatus	50.00	1	0.78						
Larus ridibundus	50.00	1	0.78						
Sterna hirundo				4.40	1	0.25	10.4	1	0.9
Falco sparverius				5.00	1	0.42	10.1	1	0.7

[a] For common names, see Appendix B

[b] Number of tests

[c] Proportion of ranked species

TABLE 3-2

Geometric Means of NOAELs, LOAELs and FEL Values for Developmental Effects from Field Studies of Birds Exposed to Dioxin-like Compounds (TEQs as µg/kg of Egg)

Species[a]	NOAEL	n[b]	Prop.[c]	LOAEL	n[b]	Prop.[c]	FEL	n[b]	Prop.[c]
Aix sponsa	0.005	1	0.1	0.02	1	0.125			
Ardea herodias	0.013	2	0.3	0.1	1	0.375	0.52	1	0.167
Pandion haliaetus	0.14	1	0.5						
Sterna forsteri	0.35	2	0.7				2.18	1	0.83
Phalacrocorax auritus				0.35	1	0.62			
Sterna caspia	1.44	1	0.9	1.42	3	0.875	2.07	1	0.5

[a] For common names, see Appendix B

[b] Number of tests

[c] Proportion of ranked species

appears to be the most sensitive species in the field, while the three anseriform species tested in the laboratory are insensitive.

Ecological similarity may also be important. Giesy and Kannan (1998) suggested that piscivorous birds are less sensitive to dioxin-like compounds than terrestrial birds such as chickens. The evidence for this generalization is weak, but suggestive (Tables 2-2 and 3-1).

3.4. EXTRAPOLATION FACTORS

Ecotoxicological test endpoints may be divided by a factor to account for the potential sensitivity of untested species. A factor of 10 is often used, based on the use of a factor of ten to account for interspecies differences in calculating reference doses for humans. The guidance for Great Lakes wildlife criteria recommends applying a factor in the range 1 to 10 to the most sensitive species, if reproductive or developmental data are available for multiple species (U.S. EPA, 1993). However, the draft guidance for soil screening levels for wildlife does not recommend a factor for interspecies differences for any chemicals (U.S. EPA, 1996). Giesy and Kannan (1998) recommend using chicken data for dioxin-like chemicals without an interspecies factor. Hence, a factor may be applied to chicken responses if a high certainty of protection is required (e.g., an endangered species is potentially exposed), particularly if the endpoint species belongs to an untested avian order. If the most similar species is used, a factor in the range 1 to 10 may be applied, depending on the degree of similarity, to account for the variance within the taxon.

Factors may also be used to extrapolate between life stages, exposure durations, and types of response. However, the body of research and testing supports the premise that embryo development is the critical response in the critical avian life stage for dioxin-like chemicals. Therefore, no factor is recommended for those considerations.

3.5. ALLOMETRIC SCALING

Allometric scaling is the adjustment of physiological, pharmacological or toxicological effective levels based on some dimension of the organisms. The most common practice is to use weight to the 0.66 or 0.75 power to scale to metabolism, which adjusts for the fact that smaller organisms tend to metabolize and excrete chemicals more rapidly. Recent studies have found that these fractional exponents do not apply to birds for many classes of chemicals, and smaller species may be more sensitive to some classes such as organophosphate pesticides (Mineau et al., 1996; Sample and Arenal, 1999). Finally, those allometric scaling models would be inappropriate for the egg exposures used in this report.

3.6. SPECIES SENSITIVITY DISTRIBUTIONS

Species sensitivity distributions (SSDs) are exposure-response relationships that represent the distribution of species sensitivities relative to exposure. SSDs are analogous to the distributions of sensitivities of individuals in conventional exposure-response relationships. Because the variance among species in sensitivity to chemicals is often more important to ecological risk assessments than variance among individuals, SSDs have become a common ecological effects model in the U.S., Europe and elsewhere (Posthuma et al., 2002).

3.6.1. Uses of Species Sensitivity Distributions. SSDs may be used in a variety of ways. First, they may be used heuristically to display the distributions of species sensitivities to assist interpretation of a multi-species data set. That is, they may serve simply as a visual summary of the data that facilitates understanding of the range of values that the effective concentrations may assume for an individual species or how an avian guild (e.g., birds that feed on soil invertebrates) or community (e.g., all birds feeding from a contaminated lake) may respond.

Second, SSDs may be used quantitatively to estimate the proportion of a taxon (e.g., herons), trophic group (e.g., piscivorous birds) or community that will be affected by an exposure (Suter et al., 2002). This is equivalent to using a conventional dose-

response function to estimate the proportion of a population that will be affected. It requires fitting some function to the SSD so that, as in other exposure-response models, the response can be estimated from the exposure level. The most common functions are the log normal or its linearized version the log probit and the log logistic or its linearized version the log logit. However, one might simply use the empirical relationship, and linearly interpolate between the points. The use of tested species to represent communities relies on the assumption that the tested species are an unbiased sample of the community. Test species are not chosen randomly, but, since species sensitivities are not known prior to testing, there is no reason to expect that the selection is biased. However, some avian families are absent from the set. This approach is common in aquatic ecological risk assessment, where endpoints are often chosen at the community level. However, endpoints for avian risk assessments are seldom defined at the community level.

Third, SSDs may be used quantitatively to estimate the probability that a species will be affected by an exposure (Suter et al., 2002). This use is more consistent with practices in avian risk assessments where the focus has been on species populations rather than taxa or communities. It is equivalent to using conventional dose-response models to estimate the individual risks (i.e., the probability that an individual will experience cancer or some other effect at a given dose) in human health risk assessments. The models are the same as those used for estimating community effects, but the effects scale is interpreted as the probability of effects on a species rather than the proportion of species affected. The underlying concept is that we do not know the sensitivity of an untested species, but we may assume that it is a random draw from the distribution of avian species sensitivities. Like the community interpretation (above), the species interpretation of SSDs depends on the set of test species being an unbiased sample of the community or taxon from which the species is drawn.

Fourth, SSDs are used to set regulatory criteria and standards in the U.S. and many other nations (Stephan, 2002; Posthuma et al., 2002). For that purpose, a proportional effect (e.g., 0.05) is selected and the corresponding concentration (e.g., the HC_5) is estimated by inverse regression.[1] This use is mentioned here in order to make it clear that this report does not derive such values. The HC_5 values calculated here are intended only to provide a point of comparison for different SSDs or for SSDs versus other values. We could have used HC_{50} values, but, because the curves are not parallel, it is preferable to compare points in the effects range that is more of concern in risk assessments.

3.6.2. Methods for Deriving Species Sensitivity Distributions. Species sensitivity distributions (SSDs) for LD_{50}, NOAEL, LOAEL and FEL data were derived with *in ovo* laboratory and field data. If multiple acceptable NOAELs, LOAELs or FELs were available for a species, the geometric mean was used as the species value as in the derivation of U.S. Water Quality Criteria. Effect concentration data for all relevant species were ranked from the lowest to the highest. Ranks are then converted to proportions using the formula, proportion = (i-0.5)/n, where i is the rank and n is the number of species. That value is the empirical proportion of all tested species with an effective concentration less than or equal to that particular species' effective concentration. Empirical SSDs for all developmental effects and for lethal effects in laboratory tests are presented in Figures 3-1 and 3-2, respectively, and SSDs for field data are in Figure 3-3.

[1]The conventional notation is HCp where HC is hazardous concentration and p is the proportion or probability, depending on the interpretation, for which the concentration is estimated.

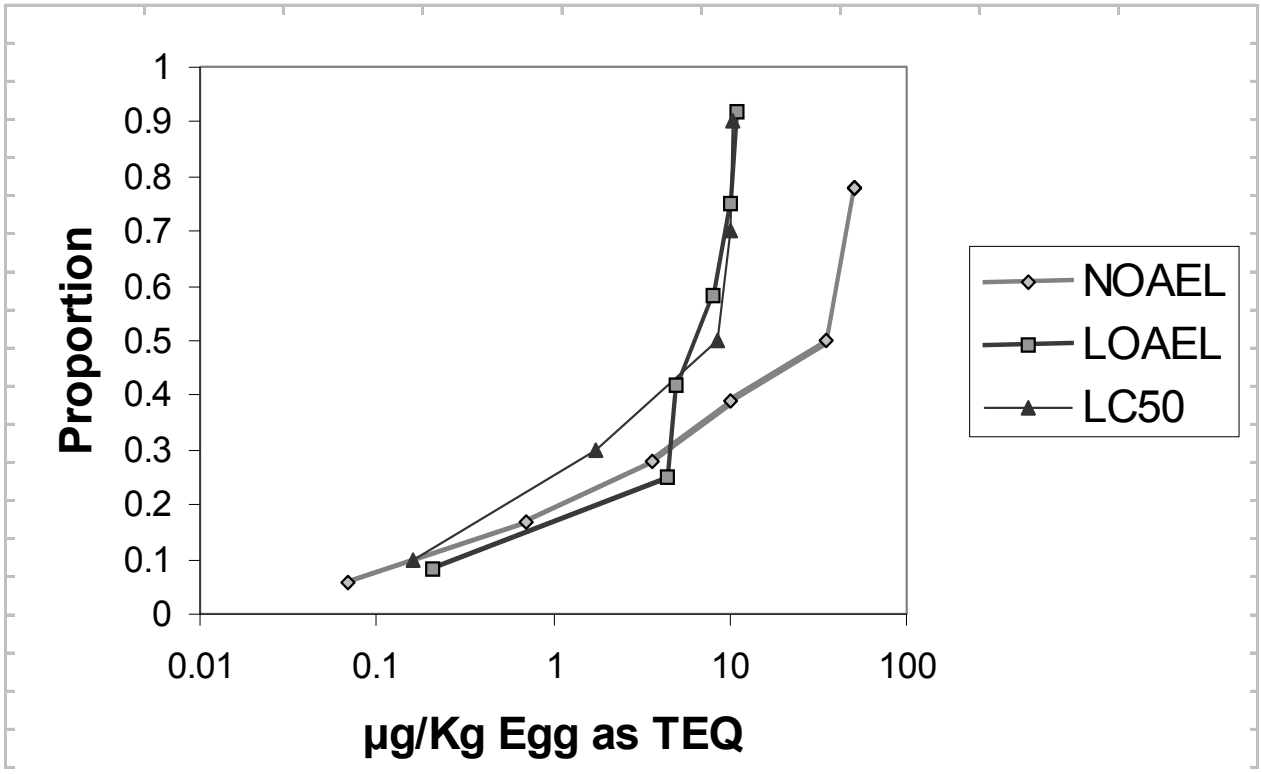

FIGURE 3-1

Empirical distribution of species sensitivity for combined lethal and sublethal developmental defects. The highest NOAEL point represents identical values for four species. The values are taken from Table 2-2 and are log-scaled

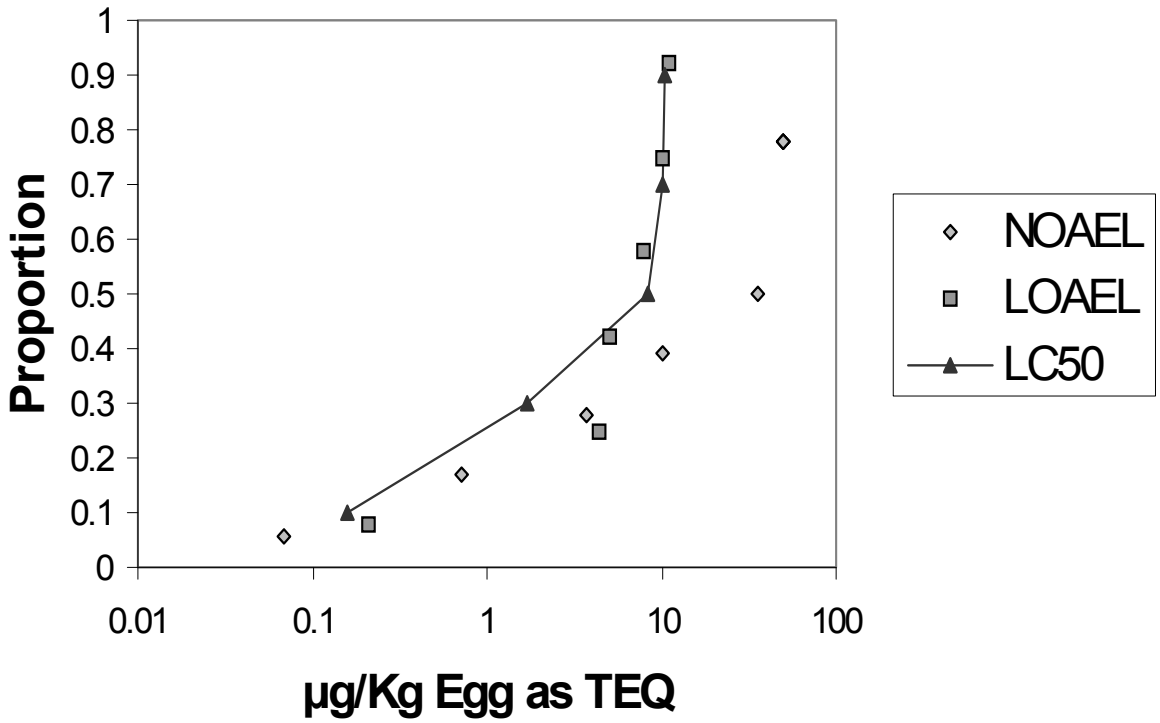

FIGURE 3-2

Empirical distribution of species sensitivity for lethal developmental effects. The highest NOAEL point represents identical values for four species. The values are taken from Table 3-1 and are log-scaled

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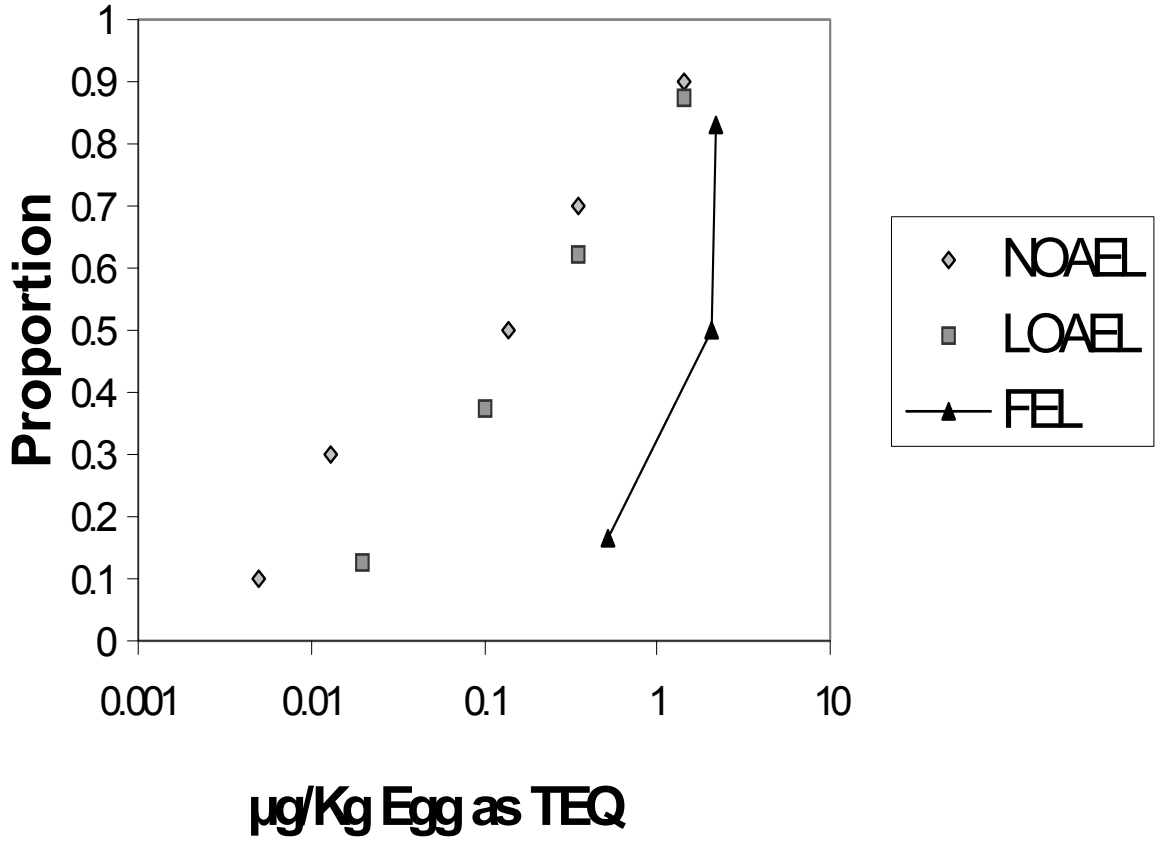

FIGURE 3-3

Empirical distribution of species sensitivity for lethal and sublethal developmental effects observed in the field. The values are taken from Table 3-2 and are log-scaled.

Models were then fit to the data (species' ranks expressed as proportions paired with corresponding species' effect concentrations) in Tables 2-2 and 3-1. The SAS General Linear Models (GLM) procedure was used to fit the log-probit, log-logit and log-weibit (the linearized Weibull) models to a preliminary data set. The log-probit and log-logit models were picked as candidate models because they are the most commonly used for SSD modeling. Although less commonly used, the Weibull model was considered because it has often been found to fit SSD data better (e.g., Jagoe and Newman, 1997; Newman et al., 2000). The differences between r^2 values for the log-probit and log-logistic models were minimal. Therefore, the log-probit model was applied to estimating HC_p values to make comparison to the general SSD literature easier because the log-probit is the most commonly applied model. Although the log-weibit model had the best fit for eight of the nine data sets as gauged by the r^2 statistic and residual plots, the improvement over the log-probit and log-logit was not sufficient to justify an unconventional model.

Most regulatory practitioners of SSD modeling recommend a minimum of five to eight observations, but Dutch standards may be derived with as few as four (e.g., Suter et al., 2002). A frequent consequence of small numbers of species is high estimation error. Newman et al. (2002) and de Zwart (2002) suggested that optimal estimation might require as many as 25 to 60 observations, but optimal data sets are seldom available for risk assessments. The number of observations in Tables 2-2, 3-1 and 3-2 ranged from 5 to 10 for the laboratory data, and 3 to 6 for the field data. Based on these low numbers of observations, the HC_p values calculated for the laboratory data do not meet most criteria for regulatory uses, but they are judged to be sufficient for screening. The HC_p values were not derived for the field studies because of the inconsistent exposures and endpoints as wells the low numbers of species. Consequently, laboratory-derived metrics were emphasized in this section of the report.

The log probit model is: $\text{Probit}(p) = a + b(\log_{10} \text{EC})$. The Probit($p$) is the probit transformation of the species proportion, EC is the effective concentration (NOAEL,

LOAEL, or LC_{50}), and *a* and *b* are the fitted intercept and slope variables, respectively. (Derivation of the species proportion is described above.) The model parameters and r^2 values are presented in Table 3-3.

These models may be used to estimate the proportion of bird species affected or the probability that a species will be affected by substituting the concentrations estimated to occur in eggs of birds at a site. They may also be used to estimate the concentrations corresponding to particular proportions or probabilities (HC_p) values. HC_p values for given values of *p* are presented in Tables 3-4 and 3-5 for all developmental effects and embryo mortality, respectively.

3.6.3. A Worked Example. This worked example summarizes the SSD model fitting process and the use of the models. The laboratory-derived NOAEL data set (Appendix A) is used for that purpose. First the SAS program converted all observations to TEQs. Next, the geometric means of the TEQs were calculated for each combination of species and test endpoint. The species TEQ geometric means were ranked from the lowest (i=1) to the highest (i=10) (Tables 2-2 and 3-1). The ranks for these 10 TEQ values were then transformed into proportions using the formula, proportion = (i-0.5)/10.

To fit a linearized lognormal (log probit) model, the $\log_{10}$ of the geometric mean of each species TEQ and the probit of the proportion are taken. The probit is the proportion expressed in units of standard deviations from the mean (normal equivalent deviation or N.E.D.) with 5 added. Most statistical programs have special functions to produce N.E.D. or probit values for any proportion. Table 7 in the appendix of Newman (1995) or similar tables in other texts also can be used for this purpose.

A linearized lognormal model is fit to the nine data pairs ($\log_{10}$ of NOAEL values versus probit of the species proportion) for embryo mortality using the SAS GLM procedure. The resulting model (see Table 3-3) is the following: Probit (proportion) =

TABLE 3-3

Log Probit Model Parameters and Squared Correlation Coefficients for Species Sensitivity Distributions Based on *In Ovo* Exposures

	Intercept (*a*)	Slope (*b*)	r^2
Combined Lethal and Sublethal Developmental Defects			
NOAEL	4.33	0.79	0.94
LOAEL	4.33	1.21	0.74
LC_{50}	4.46	1.12	0.79
Embryo Mortality			
NOAEL	4.17	0.82	0.92
LOAEL	4.23	1.28	0.70
LC_{50}	4.46	1.11	0.79

TABLE 3-4

HC_p Values for NOAELs, LOAELs and LC_{50}s Based on Developmental Effects, from Laboratory Toxicity Tests. The Values are Derived from Log Probit Models Fit to each Test Endpoint. Units are µg/kg Egg as TEQ

P	NOAEL	LOAEL	LC_{50}
0.05	0.059	0.15	0.10
0.10	0.17	0.31	0.22
0.20	0.60	0.71	0.53
0.30	1.52	1.31	1.02
0.40	3.33	2.19	1.79
0.50	6.93	3.56	3.01
0.60	14.44	5.77	5.08
0.70	31.67	9.67	8.87
0.80	79.40	17.70	17.03
0.90	283.98	40.93	42.09
0.95	813.51	81.81	88.86

TABLE 3-5

HC_p Values for NOAELs and LOAELs Based on Embryo Mortality and LC_{50}s, from Laboratory Toxicity Tests. The Values are Derived from Fitted Log Probit Models. Units are µg/kg Egg as TEQ

P	NOAEL	LOAEL	LC_{50}
0.05	0.10	0.20	0.10
0.10	0.28	0.40	0.22
0.20	0.96	0.87	0.53
0.30	2.33	1.55	1.02
0.40	4.97	2.53	1.79
0.50	10.11	3.99	3.01
0.60	20.57	6.31	5.08
0.70	43.97	10.30	8.87
0.80	106.95	18.26	17.03
0.90	366.92	40.40	42.09
0.95	1015.56	77.83	88.86

0.82($\log_{10}$ of the geometric mean of the NOAEL) + 4.17. The $\log_{10}$ HC_5 could be estimated by inserting the probit for 0.05 (i.e., 3.35515) into this equation and solving for $\log_{10}$ NOAEL. The antilogarithm of this predicted $\log_{10}$ NOAEL for the proportion of 0.05 (i.e., the antilogarithm of -0.99) is 0.10 µg/kg of egg (TEQ). Hence, HC_5 can be estimated as follows:

Probit *P*	=	4.17 + 0.82 (log HC_5)
Log HC_5	=	-0.99
HC_5	=	antilog (-0.99)
	=	0.10

For risk assessment one would estimate *P*, the proportion of species at or below the benchmark or the probability of being at or below the benchmark for a given concentration *C*. If *C* is 0.10 µg/kg egg as TEQ, the solution for the developmental failure NOAEL is as follows:

Probit *P*	=	4.17 + 0.82 (log C)
Probit *P*	=	3.35

From a table of probits or statistical software:

P	=	0.05

Hence, at 0.10 µg/kg egg as TEQ and given the model, the developmental NOAEL is exceeded for 5% of species, or the probability that the developmental NOAEL for a particular species is exceeded is 5%.

3.6.4. Results from Species Sensitivity Distributions. The chief advantage of the SSD approach is that it clearly demonstrates the wide range of sensitivities of birds to dioxin-like chemicals. A wide range of effects levels has also been observed for mammals. It also demonstrates the importance of testing a large number of species.

For example, the increase in the number of species from six for LOAELs to nine or ten for NOAELs results in an order-of-magnitude increase in the range of observed values (Tables 2-2 and 3-1) and changes the form of the SSDs (Figures 3-1 and 3-2). This is because the added species are relatively insensitive ducks, geese and gulls. Those effects of species number and selection on the distributions results in the ironic result that, for proportions greater than 0.3, the NOAELs are higher than LOAELs and median lethal levels. However, the distributions are reasonably similar for low effects levels (i.e., for $p<0.2$). If this approach were used to derive a HC_p for use as a clean-up level or other benchmark, the effects of the high NOAEL values could be eliminated by using linear interpolation or by refitting the log-probit or other function with the values above the median weighted to zero.

3.7. COMPARISON OF LABORATORY AND FIELD

As discussed above, field observations and laboratory tests provide independent estimates of the effects of dioxin-like chemicals on birds. Each has its strengths and weaknesses. Comparisons of results are difficult because of the lack of data for the same effects on the same species in the laboratory and field. The only exception is the double-crested cormorant (*Phalacrocorax auritus*). The field LOAEL for cormorant terata is 0.35 µg/kg egg TEQ, while the geometric mean LOAEL for embryo mortality in the laboratory is 14 µg/kg egg TEQ, a 40-fold difference. However, another field study of this species found that the LOAEL for EROD induction was 1.6 µg/kg egg TEQ, a 9-fold difference from the laboratory value for a nominally more sensitive endpoint. Hence, the differences between field studies for this species are nearly as large as those between laboratory and field.

Comparing the distributions of effects levels in the laboratory and field data sets provides a better basis for inference. Figure 3-4 shows the relationship between HC_5 values from laboratory SSDs (with and without chickens) and field SSDs for both

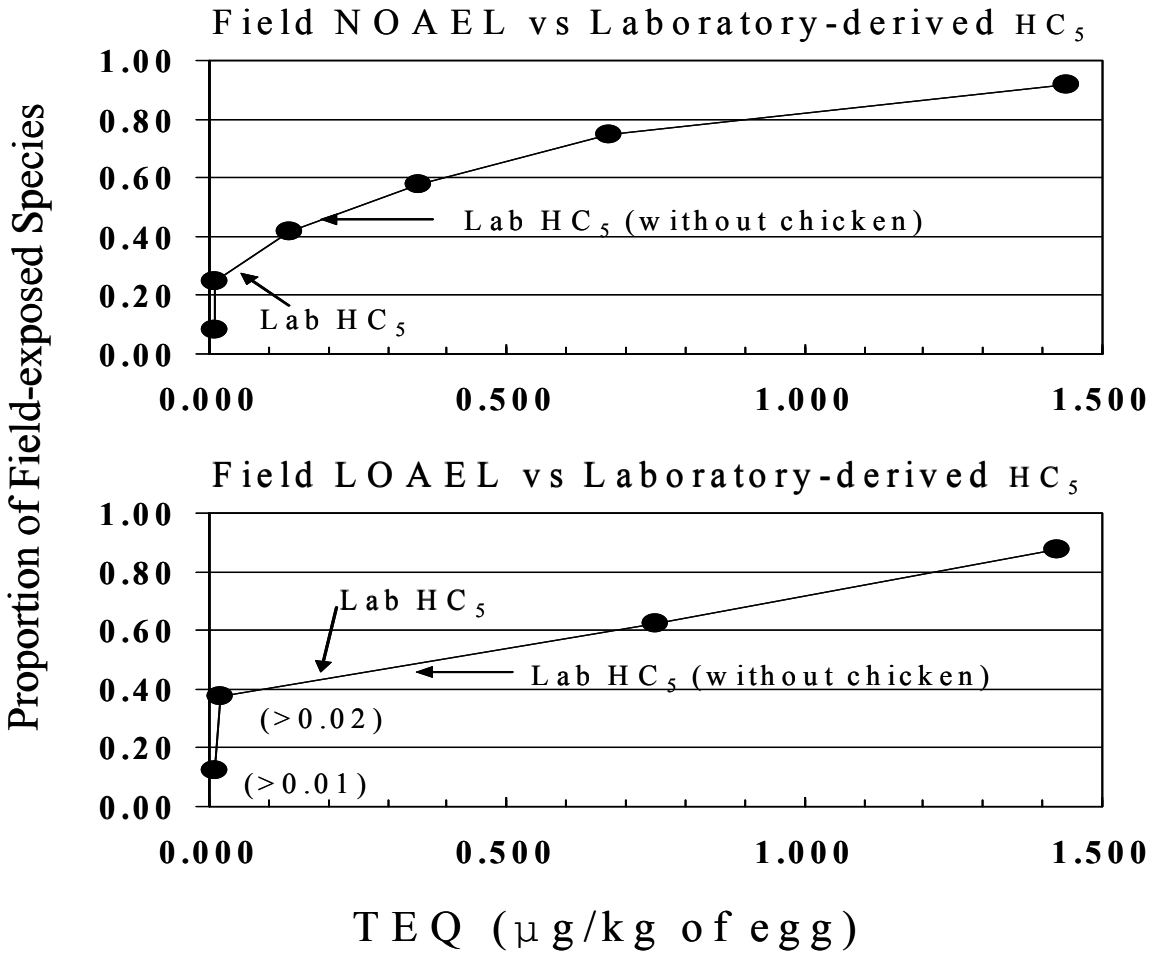

FIGURE 3-4

Comparison of HC_5 values for laboratory-derived NOAEL and LOAEL effect metrics (HC_5 indicated by arrow for models including or excluding the domestic chicken) to field-derived NOAEL and LOAEL species sensitivity distributions (dots connected by solid lines). The two smallest, field LOAEL values were “greater than” the concentration at which they were plotted.

NOAELs and LOAELs. The field effects are more sensitive, but, if chickens are included, the laboratory fifth percentiles (HC_5 values) for NOAELs and LOAELs correspond to field proportions of 27% and 40%, respectively. Hence, the discrepancies are not inordinately large, given the many differences between the laboratory and field exposures. However, without the data for chickens, the discrepancies are larger.

The presence of non-dioxin-like chemicals in field eggs seems to be the most likely explanation for the apparently greater sensitivity in the field. The authors of the field studies tried to focus on characteristic dioxin-like effects and studies were not included if other contaminants were reported to be significant concerns with respect to avian toxicity, but contributions of other contaminants could not be excluded. That is particularly the case for the most sensitive species, the wood duck (*Aix sponsa*) for which the most sensitive effect was reduced hatching success. However, other inherent differences cannot be excluded. In particular, differences in field and laboratory conditions may contribute to the greater field sensitivity. Laboratory incubators may promote the survival of embryos that might succumb in the field. Alternatively, the use of statistical significance rather than biological significance in deriving measures of effect can result in unintended biases. However, NOAELs and LOAELs should tend to be higher in field studies, because the variance is higher and the number of replicates tends to be lower than in laboratory tests. Hence, that bias would not account for the observed differences, but rather would tend to minimize them.

4. SUMMARY AND CONCLUSIONS

The critical effects of dioxin-like chemicals on birds are *in ovo* developmental effects, including deformities and mortality. The contaminant composition of eggs, from either injection or maternal contribution, is the appropriate exposure metric. This exposure may be converted to a common exposure metric, the TEQ, by TEF normalization. Such normalized concentrations in eggs were used to derive a relatively consistent data set of the comparison of different measures of effect in the laboratory and field. These measures of effect may be used with measures of exposure derived either by measuring concentrations in eggs at a contaminated site or by modeling egg concentrations to characterize avian risks from a single dioxin-like chemical or a mixture of such chemicals.

The applicability of the available avian effects data to assessments of specific species and communities were considered using alternative approaches. Because none of these methods has been endorsed by the U.S. EPA as best for wildlife risk assessments, and each has been used by the Agency in some assessments, they are simply presented here without recommendation. Risk assessors should consult with the relevant risk manager before selecting and using a method for deriving screening benchmarks.

A conclusion of these analyses is that the domestic chicken is, as is generally recognized, the most sensitive tested species, but it is not aberrantly sensitive. Given the wide range of sensitivities within birds and within mammals to dioxin-like chemicals, test data for chickens should be used.

As in most effects analyses for ecological risk assessment, a major conclusion of this report is that more data are needed. As discussed in Section 3.6.3, the small number of species tested relative to the range of avian taxa that may be exposed and the differences in the number of species for each test endpoint complicate comparisons. Some major avian taxa are conspicuously absent. These data deficiencies are common

to all data analyses, but are most conspicuous when SSDs are derived, because they reveal the size of the data set and data patterns that are not apparent when only the most sensitive or most similar species is used. The quality and consistency, as well as the number of data, are problems which make differences among species and test endpoints hard to interpret. The data set might be expanded somewhat by including publications other than peer-reviewed journals and some species may have been missed due to the emphasis on aquatic birds in the original searches. However, the problem must be solved by more consistent, high quality, peer-reviewed studies.

An advantage of the SSD approach is that it is less sensitive to moderately small data sets like that for dioxin-like effects (e.g., 4-10 species) than the conventional use of the most sensitive tested species. If, for example, there are values for a particular response in six species, it is unlikely that the most sensitive of those species is the most sensitive bird. However, if the model fit to those values is a good representation of the underlying distribution of sensitivity, then we can estimate any percentile of the distribution.

One commonly expressed concern in ecotoxicological risk assessment is that toxicity tests are more sensitive than field effects. This does not appear to be the case for avian effects of dioxin-like chemicals. The field studies analyzed here tended to yield effects at lower concentrations than the laboratory tests. This difference may be due to the presence of toxic contaminants other than dioxin-like chemicals or to other field conditions. Hence, to assess risks from dioxin-like chemicals in the field given the background of co-contaminants and imperfect parental incubation, the field data may be used as effects estimates. To assess effects of dioxin-like chemicals per se, the laboratory data should be used.

One lesson from this analysis and the prior reviews is that, although the ecotoxicological literature on dioxin-like chemicals is voluminous, relatively little of it is useful for risk assessment. Many of the studies have only one or a few exposure levels, the exposures are poorly specified, the statistics are inappropriate, the effects are not

demonstrably adverse, and other problems. A few more well-conducted studies with new species might significantly change the results of all of the approaches presented. In addition, there are no generally accepted standard protocols for egg injection studies or for field studies of reproductive effects in birds. For example, eggs may be injected in the yolk or air sac and the test chemical may be diluted in any carrier. Hence, there is extraneous variance in the data used here due to differences in the way that even the best studies are conducted, their endpoints are defined, and their data are analyzed.

One way to improve this and similar analyses would be to derive consistent test endpoints from the published studies rather than using the various endpoints reported by the authors. The assortment of NOAEC, LOAEC, LC_{50}, and FELs obscures the underlying exposure response relationships. In addition, the test endpoints based on hypothesis testing statistics do not indicate any particular effect level and are influenced by test design and performance as much as by biological response. A standard response metric might be the proportion of eggs producing normal chicks surviving at least two days post-hatch. A similar standard reproductive metric (weight of juveniles per egg) has been used successfully in analysis of chronic tests of fish (Suter et al., 1987).

In sum, the results presented here provide a defensible basis for screening ecological risk assessments of dioxin-like effects on birds. Such assessments are sufficient if exposure levels are found to be clearly in the toxic or non-toxic ranges. Where risks are marginal, it may be desirable to perform tests of the site-specific mixture. If that is not possible, the risk characterization must be performed by qualified experts to ensure proper interpretation of the results presented here in the context of the available science concerning dioxin-like toxicity.

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APPENDIX A

Laboratory and Field Data Sets for Effect of Dioxin-Like Chemicals on Avian Development from *In Ovo* Exposures

The first two tables in this appendix contain the data used in this report. They are a subset of the data contained in U.S. EPA (2001, 2002). Those reports also contain descriptions of the studies. Effects other than mortality (including failure to hatch) and developmental defects were deleted. For NOAELs and LOAELs, only the value for the most sensitive response within a study was retained. The full data sets in Tables A-1 and A-2 are referred to in the text as the developmental effects data. The mortality data set was obtained by further editing these data sets to remove nonlethal effects.

Table A-1. Laboratory data used in the analyses for this report. VALUE is the NOAEL, LOAEL, or LC50 value in µg/kg egg, as concentration of the tested compound. LVALUE is log(VALUE*TEF), so it is the log of the TEQ value.

Binomial	Chemical	LValue	Value	TEF	Effect*	Endpoint	Reference
Anas platyrhynchos	PCB77	0.69897	100.00	0.05000	EMBRYMOR	NOAEL	Brunström and Reutergardh, 1986
Anas platyrhynchos	PCB77	2.39794	5000.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Anser anser	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Brucephala clangula	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	NOAEL	Brunström and Reutergardh, 1986
Falco sparverius	PCB126	-0.63827	2.30	0.10000	TERAT/ED	NOAEL	Hoffman et al., 1998
Gallus domesticus	2378TCDD	-1.22185	0.06	1.00000	HATCHWT	NOAEL	Henshel et al., 1997a
Gallus domesticus	2378TCDD	-1.09691	0.08	1.00000	EMBRYMOR	NOAEL	Powell et al., 1996a
Gallus domesticus	2378TCDD	-1.00000	0.10	1.00000	HATCHWT	NOAEL	Henshel et al., 1997a
Gallus domesticus	PCB105	-2.00000	100.00	0.00010	EMBRYMOR	NOAEL	Brunström, 1990
Gallus domesticus	PCB105	-2.00000	100.00	0.00010	WGT	NOAEL	Powell et al., 1996b
Gallus domesticus	PCB118	-1.69897	2000.00	0.00001	EMBRYMOR	NOAEL	Brunström, 1989
Gallus domesticus	PCB126	-1.52288	0.30	0.10000	WGT	NOAEL	Powell et al., 1996b
Gallus domesticus	PCB126	-1.30103	0.50	0.10000	EMBRYMOR	NOAEL	Powell et al., 1996a
Gallus domesticus	PCB126	-1.30103	0.50	0.10000	EMBRYMOR	NOAEL	Zhao et al., 1997
Gallus domesticus	PCB126	-1.22185	0.60	0.10000	EMBRYMOR	NOAEL	Brunström and Andersson, 1988
Gallus domesticus	PCB126	-1.04576	0.90	0.10000	BRAINSYM	NOAEL	Lipsitz et al., 1997
Gallus domesticus	PCB126	-0.79588	1.60	0.10000	EMBRYMOR	NOAEL	Powell et al., 1996a
Gallus domesticus	PCB126	-0.79588	1.60	0.10000	TERAT	NOAEL	Powell et al., 1996a

Binomial	Chemical	LValue	Value	TEF	Effect*	Endpoint	Reference
Gallus domesticus	PCB126	-0.69897	2.00	0.10000	EMBRYMOR	NOAEL	Brunström et al., 1990
Gallus domesticus	PCB156	-2.00000	100.00	0.00010	EMBRYMOR	NOAEL	Brunström, 1990
Gallus domesticus	PCB157	-2.00000	100.00	0.00010	EMBRYMOR	NOAEL	Brunström, 1990
Gallus domesticus	PCB167	-1.30103	5000.00	0.00001	EMBRYMOR	NOAEL	Brunström, 1990
Gallus domesticus	PCB169	-1.52288	30.00	0.00100	EMBRYMOR	NOAEL	Brunström and Andersson, 1988
Gallus domesticus	PCB169	0.00432	1010.00	0.00100	EMBRYMOR	NOAEL	Brunström et al., 1990
Gallus domesticus	PCB77	-1.30103	1.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Gallus domesticus	PCB77	-1.30103	1.00	0.05000	WGT	NOAEL	Powell et al., 1996b
Gallus domesticus	PCB77	-1.00000	2.00	0.05000	EMBRYMOR	NOAEL	Brunström and Lund, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	MICRPHTH	NOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.34679	9.00	0.05000	BRAINSYM	NOAEL	Lipsitz et al., 1997
Larus argentatus	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	NOAEL	Brunström, 1988
Larus ridibundus	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	NOAEL	Brunström and Reutergardh, 1986
Meleagris gallopavo	PCB77	1.00000	200.00	0.05000	EMBRYMOR	NOAEL	Brunström and Lund, 1988
Phalacrocorax auritus	2378TCDD	0.00000	1.00	1.00000	EMBRYMOR	NOAEL	Powell et al., 1997a
Phalacrocorax auritus	2378TCDD	0.11394	1.30	1.00000	EMBRYMOR	NOAEL	Powell et al., 1998
Phalacrocorax auritus	PCB126	0.84510	70.00	0.10000	EMBRYMOR	NOAEL	Powell et al., 1997a
Phalacrocorax auritus	PCB126	1.30103	200.00	0.10000	EMBRYMOR	NOAEL	Powell et al., 1997b
Phasianus colchicus	2378TCDD	-1.00000	0.10	1.00000	EMBRYMOR	NOAEL	Nosek et al., 1992

Binomial	Chemical	LValue	Value	TEF	Effect*	Endpoint	Reference
Phasianus colchicus	PCB77	0.69897	100.00	0.05000	EMBRYMOR	NOAEL	Brunström and Reutergardh, 1986
Falco sparverius	PCB126	0.36173	23.00	0.10000	TERAT/ED	LOAEL	Hoffman et al., 1998
Falco sparverius	PCB77	0.69897	100.00	0.05000	EMBRYMOR	LOAEL	Hoffman et al., 1998
Gallus domesticus	2378TCDD	-2.00000	0.01	1.00000	TERAT	LOAEL	Henshel et al., 1997b
Gallus domesticus	2378TCDD	-1.00000	0.10	1.00000	HATCHWT	LOAEL	Henshel et al., 1997a
Gallus domesticus	2378TCDD	-0.79588	0.16	1.00000	EMBRYMOR	LOAEL	Powell et al., 1996a
Gallus domesticus	2378TCDD	-0.52288	0.30	1.00000	HATCHWT	LOAEL	Henshel et al., 1997a
Gallus domesticus	2378TCDD	-0.49485	0.32	1.00000	TERAT	LOAEL	Walker et al., 1997
Gallus domesticus	PCB105	-1.52288	300.00	0.00010	WGT	LOAEL	Powell et al., 1996b
Gallus domesticus	PCB105	-1.30103	500.00	0.00010	EMBRYMOR	LOAEL	Brunström, 1990
Gallus domesticus	PCB118	-1.30103	5000.00	0.00001	EMBRYMOR	LOAEL	Brunström, 1990
Gallus domesticus	PCB118	-1.09691	8000.00	0.00001	EMBRYMOR	LOAEL	Brunström, 1989
Gallus domesticus	PCB126	-1.52288	0.30	0.10000	EDEMAENZ	LOAEL	Hoffman et al., 1998
Gallus domesticus	PCB126	-1.04576	0.90	0.10000	WGT	LOAEL	Powell et al., 1996b
Gallus domesticus	PCB126	-1.00000	1.00	0.10000	EMBRYMOR	LOAEL	Powell et al., 1996a
Gallus domesticus	PCB126	-1.00000	1.00	0.10000	EMBRYMOR	LOAEL	Zhao et al., 1997
Gallus domesticus	PCB126	-0.49485	3.20	0.10000	EMBRYMOR	LOAEL	Powell et al., 1996a
Gallus domesticus	PCB126	-0.39794	4.00	0.10000	EMBRYMOR	LOAEL	Brunström et al., 1990
Gallus domesticus	PCB156	-1.30103	500.00	0.00010	EMBRYMOR	LOAEL	Brunström, 1990
Gallus domesticus	PCB157	-1.30103	500.00	0.00010	EMBRYMOR	LOAEL	Brunström, 1990
Gallus domesticus	PCB169	-1.00000	100.00	0.00100	EMBRYMOR	LOAEL	Brunström and Andersson, 1988
Gallus domesticus	PCB169	0.30535	2020.00	0.00100	EMBRYMOR	LOAEL	Brunström et al., 1990

Binomial	Chemical	LValue	Value	TEF	Effect*	Endpoint	Reference
Gallus domesticus	PCB77	-0.82391	3.00	0.05000	WGT	LOAEL	Powell et al., 1996b
Gallus domesticus	PCB77	-0.69897	4.00	0.05000	EMBRYMOR	LOAEL	Brunström and Darnerude, 1983
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.60206	5.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Gallus domesticus	PCB77	-0.30103	10.00	0.05000	EMBRYMOR	LOAEL	Brunström and Lund, 1988
Gallus domesticus	PCB77	0.00000	20.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Gallus domesticus	PCB77	0.00000	20.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Gallus domesticus	PCB77	0.00000	20.00	0.05000	EMBRYMOR	LOAEL	Brunström, 1988
Meleagris gallopavo	PCB126	0.30103	20.00	0.10000	EMBRYMOR	LOAEL	Brunström, 1989
Meleagris gallopavo	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	LOAEL	Brunström and Lund, 1988
Phalacrocorax auritus	2378TCDD	0.60206	4.00	1.00000	EMBRYMOR	LOAEL	Powell et al., 1997a
Phalacrocorax auritus	2378TCDD	0.73239	5.40	1.00000	EMBRYMOR	LOAEL	Powell et al., 1998
Phalacrocorax auritus	PCB126	1.24304	175.00	0.10000	EMBRYMOR	LOAEL	Powell et al., 1997a
Phalacrocorax auritus	PCB126	1.60206	400.00	0.10000	EMBRYMOR	LOAEL	Powell et al., 1997b
Phasianus colchicus	2378TCDD	0.00000	1.00	1.00000	EMBRYMOR	LOAEL	Nosek et al., 1992
Phasianus colchicus	2378TCDD	1.00000	10.00	1.00000	EMBRYMOR	LOAEL	Nosek et al., 1992
Phasianus colchicus	PCB77	1.69897	1000.00	0.05000	EMBRYMOR	LOAEL	Brunström and Reutergardh, 1986
Sterna hirundo	PCB126	0.64345	44.00	0.10000	EMBRYMOR	LOAEL	Hoffman et al., 1998
Falco sparverius	PCB126	0.81291	65.00	0.100	EMBRYMOR	LC50	Hoffman et al., 1998
Falco sparverius	PCB77	1.19866	316.00	0.050	EMBRYMOR	LC50	Hoffman et al., 1998
Gallus domesticus	PCB126	-1.39794	0.40	0.100	EMBRYMOR	LC50	Hoffman et al., 1998

Binomial	Chemical	LValue	Value	TEF	Effect*	Endpoint	Reference
Gallus domesticus	PCB126	-0.50864	3.10	0.100	EMBRYMOR	LC50	Brunström and Andersson, 1988
Gallus domesticus	PCB169	-0.76955	170.00	0.001	EMBRYMOR	LC50	Brunström and Andersson, 1988
Gallus domesticus	PCB77	-0.88606	2.60	0.050	EMBRYMOR	LC50	Hoffman et al., 1998
Gallus domesticus	PCB77	-0.36653	8.60	0.050	EMBRYMOR	LC50	Brunström and Andersson, 1988
Phalacrocorax auritus	2378TCDD	0.60206	4.00	1.000	EMBRYMOR	LC50	Powell et al., 1998
Phalacrocorax auritus	PCB126	1.24797	177.00	0.100	EMBRYMOR	LC50	Powell et al., 1998
Phasianus colchicus	2378TCDD	0.13033	1.35	1.000	EMBRYMOR	LC50	Nosek et al., 1992
Phasianus colchicus	2378TCDD	0.33846	2.18	1.000	EMBRYMOR	LC50	Nosek et al., 1992
Sterna hirundo	PCB126	1.01703	104.00	0.100	EMBRYMOR	LC50	Hoffman et al., 1998

*Effect codes are defined in Table A-3.

Table A-2. Field data set used for the analyses in this report. Value is the NOAEL, LOAEL, or FEL in µg/kg egg as TEQ.

Chemical	Binomial	Effect*	Value	Endpoint	Reference
PCDDPCDF	*Ardea herodias*	TERAT/FLEDG	0.5190	FEL	Hart et al., 1991
PCDDPCDF	*Ardea herodias*	TERAT/FLEDG	0.0176	NOAEL	Hart et al., 1991
PCDDPCDF	*Ardea herodias*	BRAINSYM	0.100	LOAEL	Henshel et al., 1995
PCDDPCDF	*Ardea herodias*	BRAINSYM	0.0100	NOAEL	Henshel et al., 1995
PCDDPCDF	*Pandion halieatus*	HATCH/FLEDG	0.1360	NOAEL	Woodford et al., 1998
PCDDPCDF	*Aix sponsa*	REPROD	0.0200	LOAEL	White and Seginak, 1994
PCDDPCDF	*Aix sponsa*	REPROD	0.0050	NOAEL	White and Seginak, 1994
PCBS	*Sterna forsteri*	HATCH/FLEDG	2.1750	FEL	Kubiak et al., 1989
PCBS	*Sterna forsteri*	HATCH/FLEDG	0.2010	NOAEL	Kubiak et al., 1989
PCBS	*Sterna forsteri*	FLEDGING	0.6110	NOAEL	Harris et al., 1993
PCBS	*Sterna caspia*	WASTING	1.6000	LOAEL	Ewins et al., 1994
PCBS	*Sterna caspia*	WASTING	1.4400	NOAEL	Ewins et al., 1994
PCBS	*Sterna caspia*	HATCH/FLEDG	1.3900	LOAEL	Ludwig et al., 1993
PCBS	*Sterna caspia*	HATCH/FLEDG	2.0700	FEL	Ludwig et al., 1993
PCBS	*Sterna caspia*	TERATA	1.3000	LOAEL	Yamashita et al., 1993
PCBS	*Phalacrocorax auritius*	TERATA	0.3500	LOAEL	Yamashita et al., 1993

*Effect codes are defined in Table A-3

Table A-3. Effect codes used in Tables A-1 and A-2 and the corresponding effects.

Effect Code	Effect
BRAINSYM	Brain asymmetry
EDEMAENZ	Edema and enzyme induction
EMBRYMOR	Embryo mortality
HATCH/FLEDG	Reduced hatching and fledging
HATCHWT	Reduced weight at hatching
NOFLEGING	No successful fledging
MICRPHTH	Microphthalmia
REPROD	Reduced reproductive success
TERAT	Terata
TERAT/ED	Terata and edema
TERAT/FLEDG	Terata and reduced fledging
WASTING	Wasting syndrom
WGT	Weight of hatchlings

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APPENDIX B

Scientific and Common Names of Birds

Aix sponsa	Wood duck
Anas platyrhyncus	Mallard
Anser anser	Greylag goose
Ardea herodius	Great blue heron
Bucephala clanga	Common goldeneye
Gallus domesticus	Chicken
Larus argentatus	Herring gull
Larus ridibundus	Black-headed gull
Meleagris gallopavo	Turkey
Pandion haliaetus	Osprey
Phalacrocorax auritus	Double-crested cormorant
Phasianus colchicus	Ring-necked pheasant
Sterna caspia	Caspian tern
Sterna forsteri	Forster's tern
Sterna hirundo	Common tern