CHAPTER 2

Exposure, Uptake, and Disposition of Chemicals in Reproductive and Developmental Stages of Selected Oviparous Vertebrates

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Chemical exposure is defined here as the delivery of the chemical from the surrounding environment into the organism and transport to the active site. The actual time-variable dose delivered to the active site depends in complex ways on the physicochemical speciation of the chemical outside and inside the organism and upon the behavior, bioenergetics, physiology, and life stage of the organism. Once a chemical enters an organism, it may be presented to the active site, excreted, or sequestered within tissue away from the active site. Whether a stored chemical is eventually redistributed to the active site (and completes the exposure pathway) or is metabolized, excreted, or passed to offspring depends largely upon the physiology of the organism. Such storage within the organism may serve to delay exposure, resulting in a temporal (and perhaps spatial) disconnection between external exposure and observed effect.

In another light, exposure can also be described as the result of a organism's being physically located in an area that contains the chemical in the appropriate (e.g., bioavailable) form. Taken this way, quantifying exposure then becomes a probability exercise based on the spatial and temporal distribution of bioavailable chemical and the behavior and physiology of the organism. This approach allows for time-variable exposure calculations. For example, exposure to a persistent, globally produced organochlorine such as *p,p'*-DDE is likely to be low and fairly uniform spatially and temporally. In contrast, exposure to a nonpersistent, high-use chemical such as an organophosphorus agrochemical may be high but short-lived in a restricted region. The ultimate impact of a chronic, continual, low-level exposure versus a large but episodic exposure is an interesting ecotoxicological question.

CHAPTER PREVIEW

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Exposure assessment often begins with the characterization of chemical release during production, transport, processing, end-use, and disposal. The extent of release will generally depend upon such factors as the production volume and physicochemical properties of the chemical, the nature of industrial application (i.e., open or closed system), availability, type and operational features of pollution control equipment, and end-use and ultimate disposal patterns of products that contain the chemical. Natural emission sources may constitute an important component of the overall emission inventory of certain chemicals, and therefore also should be considered.

Upon release into the environment, contaminants are subjected to various environmental transport and transformation processes that determine the spatial and temporal concentrations in primary (air, water, soil, sediment) and secondary (plants, animals) compartments. The concentrations in these media, when linked to species and life-stage-specific exposure factors of oviparous organisms (i.e., inhalation rates, ingestion rates, exposure patterns), dictate the total administered dose to a given population of receptors from multiple exposure pathways. The efficiency in which the administered dose is absorbed across the respiratory, gut, or skin interface determines the absorbed dose. Further consideration of toxicokinetic behavior of the chemical determines tissue residues and the effective dose at the internal target site which, in turn, mediates adverse biological effects.

For most organisms, early developmental stages are among the most sensitive to the toxic effects of chemical contaminants. Success in terms of reproduction and development represents the integration and ontogenetic development of many critical biochemical events. Chemical interference with these events may lead to toxicological effects that influence the survival of the individual and possibly populations. In addition to sensitive sites for toxicity, the process of ontogeny brings about changes in how the organism is exposed to chemicals, how significant the exposure is, and how the organism deals with the chemicals once they are absorbed. In a true sense the organism undergoes an exposure and dispositional ontogeny. All of these events are temporally spaced, presenting windows of opportunity for xenobiotics to more capably elicit their toxic effects. Because of differences in the relative importance of exposure pathways for the various life stages, extrapolation of exposure estimates from adults of even the same species are of little value.

Oviparous species present a number of unique considerations with regard to chemical exposure, uptake, and disposition in early life stages. By definition these animals undertake their early development in eggs outside of the maternal body. The egg, more or less a self-contained unit, stores yolk nutrients, provided from maternal sources, that serve to fuel growth and development. This confinement of early development in the egg modulates chemical exposure from the outside but also subjects the embryo to chemicals that may be preferentially mobilized or concentrated from maternal sources. Furthermore, the early life stages are free of maternal influences with regard to xenobiotic disposition. Low biotransformational

activities and incomplete excretory pathways dictate unusual toxicological conditions in developing stages of oviparous organisms.

In this chapter we explore the major pathways resulting in exposure of a wide variety of chemicals to oviparous vertebrates, with emphasis on critical life stages for reproduction and development. First, we discuss "external processes"—those pathways that deliver chemicals to the organism. Then we discuss "internal processes"—the partitioning, redistribution, and disposition of chemicals within the organism, including maternal transfer. Finally, we discuss modeling approaches to xenobiotics in oviparous species. While fundamental processes influencing chemical speciation and bioavailability apply to all chemical classes, we have based our discussions mainly on persistent organic chemicals and metals. Similarly, chemical exposure, uptake, and disposition is discussed predominately for fish and birds. Such an emphasis is not a reflection of perceived importance of these groups; rather, it reflects the existing literature base and the limitations dictated by the format of the workshop from which this chapter arises. By design this chapter is chemically oriented, leaving toxicological considerations to later discussions in this volume.

External Factors Controlling the Magnitude and Variability of Exposure

The challenge of quantifying exposure of a wide variety of chemicals

The extremely wide variety of chemicals produced and used in many different applications compromises our ability to generalize about the processes controlling their exposure to oviparous organisms. To illustrate this variability here, we review the production, use, and physical properties of several classes of environmentally relevant chemicals.

Types and quantities of releases to the environment

In order for a chemical to exert a significant risk to a population, it must be present at the receptor site at high enough levels and for a long enough duration to cause an effect. Therefore, one way to rank the relative "threat" of various chemicals is to quantify 3 independent parameters: production, persistence, and potency. "Production" is defined here as the gross rate at which the chemical is manufactured, modified by the fraction of production that is released into the environment. Production of chemicals of environmental interest range from those produced in extremely small quantities (i.e., 2,3,7,8-tetrachlorodibenzodioxin [TCDD]) to those produced in extremely large quantities (petroleum products, surfactants, structural polymers). Likewise, the fraction of gross production released to the environment ranges from near zero (i.e., fuel grade plutonium) to 100% (agrochemicals). Production and release rates, however, are insufficient to assess potential risk, as chemicals released into the environment vary considerably in their persistence. Only those

chemicals that resist abiotic and biotic degradation processes and are mobile can be transported to potentially vulnerable populations. Again, the wide variety of chemicals that have been hypothesized to cause harm to oviparous organisms have an incredible range of environmental half-lives, ranging from less than 1 day to decades or centuries. Finally, one must consider the potency of the chemical on the target receptor species. While a detailed discussion of the large number of possible "effects" mechanisms is beyond the scope of this chapter, suffice it to say that chemicals display a very wide range of modes of action and of potencies. The large variety in production, persistence, and potency across the chemicals, which may impact oviparous organisms, is evidence that one cannot generalize the environmental behaviors and exposure pathways of these chemicals. Developing quantitative models with suitable plasticity to describe such widely varying behavior is a major challenge.

Uses, properties, and environmental persistence of the chemicals

A wide range of chemicals, both anthropogenic and naturally occurring, have the potential to cause reproductive and developmental effects. In order to address the questions about both the external and internal exposure concentrations to these chemicals, we first need to know what chemicals we are dealing with, their physicochemical properties, chemical structures, rates of degradation in the environment and elimination rates in biota. We need similar information for possible biologically active degradation products. Table 2-1 contains data information for assessment of external and internal doses for 42 chemicals. These chemicals are of potential toxicological importance for reproduction and development as determined by recent reviews (Soto et al. 1995; GEA 1996; USEPA 1997) and papers contained within this volume. This list, which is by no means comprehensive, contains chemicals that are very diverse in their properties, and hence the potential sinks of active compounds range widely. Chemicals in Table 2-1 range from relatively hydrophilic (log K_{ow} < 1: amitrole) to hydrophobic (log K_{ow} = 6.8: TCDD). Structurally these chemicals range from low-molecular-weight, cyclic aliphatics (e.g., amitrole, MW = 84 Daltons) to oligomers (e.g., nonylphenol polyethoxylates). The properties of selected chemicals, important to show the breadth of chemicals involved or important to subsequent topics, are discussed in further detail in the following text. By overlaying the compounds' fate and exposure profile with known reproductive and developmental endpoints, an assessor may begin to determine potential exposure to various life stages of organisms.

Persistent organochlorines

Persistent organochlorines include many subclasses of compounds; however, the overall environmental fate characteristics are similar. Basically the fate characteristics of organochlorines are predominantly described by the persistence of the parent or parent-like compound and by their hydrophobic tendencies. Because of the ubiquitousness of the individual organochlorines throughout terrestrial and aquatic

Table 2-1 Physicochemical properties and chemical fate data for chemicals with reputed reproductive or developmental effects

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment[e] DT50 (days)	Water/sewage[f] DT50 (days)	Microbial[g] biodegrad-ability	Biologically active deg'n product?	Reference
Trifluralin	Dinitroaniline	H	mitosis inhibitor	$9–11\times10^3$	335.3	3.97	3.8	Neutral	60 (60–132)	< 1 (photolysis)	+++	Aniline dealkylation pdt	1,2,3
Atrazine	Chlorotriazine	H	—	$28–30\times10^3$	215.7	2.5	0.15	1.7	60(45–119)	> 100	+	De-ethyl atrazine	1,2,3
Amitrol	Aminotriazole	H	thyroid tumors	—	84.1	< 1	0.1	Neutral	14(4–23)	~70	++		1,2
Benomyl	Carbamate	F	tetrato-genicity	4.8×10^2	290.4	1.1	1.9	Neutral	67(10–356)	< 1	+++	5-OH-benzimidazole carbamate	1,2,3
Iprodione	Carboxamide	F	develop-mental toxicity at high doses	4.3×10^2	330.2	3	0.7	Neutral	14(<7–160)	?	+++	Similar to vinclozolin	1,3
Mancozeb	Ethylene bisdithio-carbamate	F	thyroid tumors	$1.8–3.2\times10^3$	polymer	?	> 2	Neutral	70(7–139)	1–2	+++	Ethylene thiourea	1,3
Ethylene thiourea	Thiourea	F	thyroid tumors	—	102	–0.66	–1.0	Neutral	14(7–28)	?	++		1
Metiram	Dithiocarb-amate-Zn-dithione	F	thyroid tumors	4.2×10^2	1089	2	500	Neutral	20	?	+++		1,3
Tributyl tin oxide	Organo-metallic	F	imposex	—	289.7	3.8	600	pKOH ~7	> 300	7(2–14)	+	mono-, dibutyltins	4

Table 2-1 continued

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment [e]DT50 (days)	Water/ sewage[f] DT50 (days)	Microbial[g] biodegrad-ability	Biologically active deg'n product?	Reference
Vinclozolin	Dicarboximide	F	deg'n pdt antiandro-genic	54	286.1	3	0.1	Neutral	20(3–75)	?	+++	Hydrolysis deg'n pdt	3
Diflubenzuron	Benzamide	I	chitin growth inhibitor	35	310.7	3.89	10	Neutral	10(3–60)	?	++++	OH-metabolite	1,3
Azadirachtin	Tetranortri-terpenoid	I	ecdysone blocker	—	720.7	1.09		Neutral	< 10	< 10	++++		5
Fenoxycarb	Phenoxycarba-mate	I	molt inhibitor	—	301.3	4.07	1	Neutral	1(1–31)	?	++++	OH-metabolite	1,3
Carbaryl	Naphthyl-carbamate	I	AchE inhibitor	$0.9–1.8\times10^3$	201.2	2.36	0.38	Neutral	10(4–22)	12–30	+++	1-naphthol & OH-metabolites	1,3
Methomyl	Thioaceti-midate	I	AchE inhibitor	7.8×10^2	162.2	0.2	0.16	Neutral	30(8–45)	< 30?	++		1,3
Parathion	Nitrophenyl phosphoro-thioate	I	AchE inhibitor	$1.8–3.2\times10^3$	291.3	3.9	1.5–15	Neutral	14(7–35)	< 7	++	p-nitrophenol, aminoparathion	1,3

Table 2-1 continued

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment[e] DT50 (days)	Water/ sewage[f] DT50 (days)	Microbial[g] biodegrad-ability	Biologically active deg'n product?	Reference
Dicofol	Chlorophenyl trichloro-ethanol	I	weak estrogen	4.9×10^2	370.5	4.7	5	Neutral	45(40–50)	15–93	++	Dichloro-benzophenone	1,3
Dieldrin/ aldrin	Chlorinated cyclic aliphatic	I,M	weak estrogen	NP	380.9	5.4	7.4–12	Neutral	> 1000	> 10	–		1,5
Endosulfan	Chlorinated cyclic aliphatic	I	weak estrogen	8.0×10^1	406.9	3.8	2.9–6.8	Neutral	50(10–200)	< 7	+	Endosulfan sulfate	1,3
Toxaphene	Chlorinated bornane/ camphene	I	weak estrogen	NP	414	6.4	210	Neutral	> 500	> 500	–	Dechlorination	1,5
Methoxychlor	Methoxy-phenol trichloroethane	I	weak estrogen	40	345.7	3.9	80–100	Neutral	120(7–210)	~46	+	O-dealkylation to phenols & OH–	1,3
p,p'-DDE	Chlorophenyl dichloroethane	M	anti-androgen	NP	318	5.7	200	Neutral	> 1000		–	deg'n pdt	1,5

Table 2-1 continued

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment [e]DT50 (days)	Water/sewage[f] DT50 (days)	Microbial[g] biodegradability	Biologically active deg'n product?	Reference
o,p'-DDT	Chlorophenyl dichloroethane	I,B	weak estrogen	NP	354.5	6	410	Neutral	> 1000		+	o,p'-DDE	1,5
Chlordecone	Chlorinated cyclic aliphatic	I	weak estrogen	NP	490.7	4.5	13	Neutral	> 1000	> 5	–		1,5
Tetrachloro-biphenyls	Chlorinated biphenyl	FR, P	adreno-corticoid, thyroid & estrogenic effects	NP	292	5.6–6.5	163–1300	Neutral	> 1000	500–900	–		6
Trichloro biphenyls	Chlorinated biphenyl	FR,P	adreno-corticoid, thyroid & estrogenic effects	NP	257.5	5.5–5.9	130–330	Neutral	> 1000	500–900	–		6
Hydroxy-tetrachloro biphenyls	Chlorinated biphenylol	M	weak estrogens, thyroid hormone mimics	NP	308	4.9–5.8	33–260	?	?	?	+++	deg'n pdt in fish, birds, and mammals	
Hydroxy-trichloro Biphenyls	Chlorinated biphenylol	M	weak estrogens, thyroid hormone mimics	NP	274.5	4.8–5.2	26–65	?	?	?	+++	deg'n pdt in fish, birds, and mammals	
2,3,7,8-TCDD	Chlorinated dibenzo-p-dioxin	B	adreno-corticoid, thyroid & estrogenic effects	NP	322	6.8	> 100	Neutral	> 1000	< 2	–		7

Table 2-1 continued

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment[e] DT50 (days)	Water/sewage[f] DT50 (days)	Microbial[g] biodegradability	Biologically active deg'n product?	Reference
p-tertbutyl hydroxy anisole	Phenolic	A	weak estrogen	—	164	3.8	2.6	Neutral				Butyl phenol	8
p-tertbutyl-phenol	Phenolic	M	weak estrogen	—	150	3.3	0.8	?					8,9
p-nonylphenol	Phenolic	S,M	weak estrogen	—	220	4.5, 5.8	13, 260	?	> 7	< 7	++		9, 10
p-octyl phenol	Phenolic	S,M	weak estrogen	—	206	5.4	100	?	> 7	< 7	++		9
Nonylphenyl ethoxylate (EO=9)	Ethoxylate oligomer	S	weak estrogen	> 242×10^3	598	−5.9, −7.2	330, 6500	Neutral	> 7	< 7	+++	Nonyl phenol	
Nonylphenol ethoxylate (EO=2)	Ethoxylate dimer	S	weak estrogen	—	304	4.4, 5.6	100, 163	Neutral	> 7	'7–21	++	Nonyl phenol	10, 11
Nonylphenol carboxylate	NPE deg'n pdt	M	weak estrogen	—	256	3.5	130	?		< 7	++		
Bis-phenol A	Hydroxy diphenyl-propane	M	weak estrogen	—	228.3	3.32	0.08	?	> 300	< 4(<1–28)	+	isopropyl phenol	9

Table 2-1 continued

Compound	Class	Use[a]	Known effects	Quantity used annually in U.S. (T)[b]	MW	Log K_{ow}[c]	K_{oc}[d] ($\times 10^3$)	pKa	Soil/sediment[e] DT50 (days)	Water/sewage[f] DT50 (days)	Microbial[g] biodegradability	Biologically active deg'n product?	Reference
4-hydroxybiphenyl	Phenolic	S,M	weak estrogen	NP	206	3.2	0.65	?	1–7	1–7	+++		9, 12
Dibutyl phthalate	Phthalate ester	P	weak estrogen	—	278.3	4.72	6.8	Neutral	~180	< 5(2–12)	+++	Phthalic acid	8, 12
Butylbenzyl phthalate	Phthalate ester	P	weak estrogen	$>50\times10^3$	312.4	4.91	0.35	Neutral	?	<2<1–7	++++	phthalic acid, benzyl alcohol	8, 12
Ethynylestradiol	Phenol	SH	synthetic estrogen	—	296	3.67	1.9	?	?	> 5	+++		9, 13
Beta-sitosterol	Phenol	PY	phyto-estrogen	—	414.7	> 5	> 40	?					9

[a]H=herbicide; F=fungicide; I=insecticide; M=metabolite; B=by product; FR=flame retardant; P=plasticizer; A=antioxidant; S=surfactant; SH=synthetic hormone; PY=phytoestrogen

[b]Dash indicates no annual production figures published in the open literature. Pesticide use information from USGS pesticide National Synthesis Project (http://water.wr.usgs.gov/pnsp/use 92). Represents approximate quantities annually used in period. NP = not deliberately produced or no longer produced in U.S. or western Europe, but residues remain in soils and sediments.

[c]K_{ow} from Howard 1991; Howard et al. 1991; Hansch et al. 1995; or calculated from fragment constants using structurally related compounds (e.g., OH-PCBs from PCBs).

[d]K_{oc} calculated from $0.41 * K_{ow}$ (Karickhoff 1981) when measured values not available.

[e]"Soil/sediment DT50" = 50% disappearance time in field studies (pesticides) or soil/sediment biodegradation tests (industrial chemicals).

[f]"Water/sewage DT50" =50% disappearance time (if available) or range of reported results for water column in field or lab studies (pesticides) or sewage sludge incubations (mainly industrial compounds).

[g]Microbial degradability: qualitative ranking from non-degradable (–) to highly degradable (++++)

References: 1) Wauchope et al. 1992; 2) Howard 1991; 3) Tomlin 1994; 4) Stewart and Mora 1990; 5) Thompson 1992; 6) Augustijn-Beckers et al. 1994; 7) Mackay et al. 1992a; 8) Mackay et al. 1992b; 9) Howard et al. 1991; 10) Hansch et al. 1995; 11) Ahel et al. 1994; 12) Kvestak and Ahel 1995 13) Budavari et al. 1996

environments, the organism appears to be subjected to assault throughout all life stages.

Polychlorinated biphenyls (PCBs): The physical properties of selected chlorobiphenyl homologues are given in Table 2-1. Most polychlorinated biphenyl (PCB) congeners, particularly those lacking adjacent unsubstituted positions on the biphenyl rings (e.g., 2,4,5-, 2,3,5-, or 2,3,6- substituted on both rings), are extremely persistent in the environment and are essentially non-biodegradable in aerobic soils or sediments (Mackay et al. 1992a). Highly chlorinated PCBs have been shown to be dechlorinated in anaerobic sediments, but only where present at relatively high concentrations (> 10 mg/g dry weight) (Brown and Bedard 1987; Rhee and Sokol 1993). PCBs also have extremely long half-lives in adult fishes. For example, an 8-year study of eels found that the half-life of PCB153 was > 10 years (de Boer et al. 1994). A large survey of freshwater fishes in U.S. rivers and lakes found PCBs were the most prominent organochlorine contaminants, with median concentrations of 209 ng/g wet weight (USEPA 1991). Penta- and tetrachlorobiphenyls were the predominant homologue groups, with median concentrations of 72 and 23 ng/g wet weight, respectively.

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans: While polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are rapidly photodegraded in air, in water, and on surfaces (Buser 1988), they are extremely hydrophobic and resistant to biodegradation in soils and sediments. Historical profiles of PCDD/Fs in sediment cores from large lakes show no evidence of transformation of congeners (such as anaerobic dechlorination) over time (Hites 1990). Dechlorination appears to be a major route of degradation of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and -TCDF in sunlight in natural waters (Dung and O'Keefe 1994). The 2,3,7,8-substituted PCDD/F congeners are known to bioaccumulate in fish and invertebrates; however, non-2,3,7,8-substituted congeners (which predominate in combustion sources) are readily degraded by vertebrates (Opperhuizen and Sijm 1990). Data on the bioavailability of TCDD from sediments and water, as well as on the pharmacokinetics in fish, are available. TCDD has a relatively long half-life in adult fishes (~ 1 year) (Kuehl et al. 1989). Surveys of PCDD/Fs in fish collected in 1986 from 388 locations in the U.S. (freshwater only) found 2,3,7,8-TCDD was detectable at 70% of all locations at median concentrations of 1.4 pg/g wet weight with maximum concentrations of 204 pg/g (USEPA 1991). Current levels may be lower because highest TCDD and 2,3,7,8-TCDF levels were found in fish near pulp and paper mills using chlorine; since then, these mills have substantially reduced TCDD emissions. The USEPA surveys found 2,3,4,7,8-pentachlorodibenzofuran (PnCDF), 1,2,3,6,7,8-hexachlorodibenzodioxin (HxCDD) and 1,2,3,4,6,7,8-heptachlorodibenzodioxin (HpCDD) were more prominent in fish tissue than was 2,3,7,8-TCDD at non-paper mill sites (median concentrations in the low pg/g wet weight range).

Toxaphene: This complex mixture of polychlorobornanes and camphenes was widely used in the U.S. on cotton crops. Toxaphene is produced by the chlorination

of technical camphene or α-pinene and can consist of over 300 congeners, mainly bornanes and camphenes substituted with 6 to 10 chlorines, with an average composition of $C_{10}H_{10}Cl_8$. Determining the environmental fate of a mixture of compounds such as toxaphene is difficult, as each structurally different compound in the mixture will have a specific set of chemical properties.

Use of toxaphene peaked between 1972 and 1975. Manufacturing was banned in the U.S. in 1982 and use ceased in 1986 (Voldner and Li 1993). Similar products have been, and may continue to be, used in Mexico, Central America, eastern Europe, and the former Soviet Union. Toxaphene is extremely persistent in soils following pest control application, with half-lives ranging from 1 to 14 years (Howard et al. 1991) (Table 2-1). Losses from soil are mainly through volatilization and runoff (Glotfelty and Taylor 1984). Toxaphene degrades mainly through dechlorination in sediments and its dechlorination products are bioavailable in lakes treated with toxaphene 30 years later (Miskimmin et al. 1995). A half-life for toxaphene of 63 days was reported for juvenile lake trout (Mayer et al. 1977) and up to 1 year in injected adult fishes (Delorme et al. 1993). Glassmeyer et al. (1997) found mean concentrations of toxaphene in lake trout in the 5 Great Lakes ranging from 140 to 3500 ng/g wet weight. Lowest concentrations were found in lake trout from Lake Erie and highest in samples from Lake Superior.

DDT:-1,1,1-trichloro-2,2-Bis (p-chlorophenyl) ethane: The technical product consists of 4,4'-DDT (or p, p'-substituted) and its *o,p*-DDT isomer, as well as their dechlorinated analogs [*p,p'*- and *o,p'*- 1,1-dichloro-2,2'-bis (p-chlorophenyl) ethane (DDD)]. Its use has been restricted in Canada, the U.S., and western Europe for nearly 2 decades; however, it is used in pest control programs in southern Asia, Africa, Mexico, and Central and South America (Voldner and Ellenton 1987) and may be used in China and Russia. DDT, especially its metabolite 1,1-dichloro-2,2' bis (p-chloro-phenyl) ethylene (*p,p'*-DDE), is extremely persistent in soils and sediments and has a long half-life in biota. *p,p'*-DDE is probably the most common individual organochlorine contaminant in aquatic and terrestrial biota, and it can be present at low mg/g wet weight levels in tissues of top predators such as fish-eating birds. A survey of fishes from 388 locations in the U.S. found *p,p'*-DDE in 98% of all samples at median concentrations of 58 ng/g wet weight with maximum levels of 14 mg/g wet weight (USEPA 1991). Levels of DDT and its principal metabolite, DDE, have decreased in fish and wildlife of western Europe, North America, and Japan in the past 15 years due to bans on use.

Current use pesticides

Carbamate/thiocarbamate pesticides: Table 2-1 includes a structurally diverse group of insecticides and fungicides reported to have developmental effects in invertebrates, fish and birds (USEPA 1997). Several are designed as inhibitors of various stages of insect development, e.g., fenoxycarb, diflubenzuron, azadirachtin. All are carbamates, substituted ureas, or thiocarbamates and therefore are readily

hydrolyzed chemically or microbially in soils and in vivo in organisms. All of these compounds are expected to have short half-lives in fish and other aquatic organisms and therefore would not biomagnify in food chains of piscivorous fish or birds. Their relatively low log K_{ow} (1 to 4) suggest they would be accumulated mainly from water. For example, diflubenzuron has been found to have a half-life of < 2 d in bullheads and < 1 d in sunfish (Niimi 1987). Direct exposure of oviparous organisms to these relatively nonpersistent chemicals could occur through spray drift or runoff from treated fields soon after application. Thus quantities used and type of application (e.g., aerial versus ground rig) may be critical aspects of exposure.

Tributyltin: Tributyltin (TBT) is a broad-spectrum algicide, miticide, fungicide, and insecticide (Stewart and de Mora 1990). TBT and other organotin compounds were first used in agriculture; subsequently, TBT has had wide application as a marine antifoulant starting in the 1960s. Its most important entry route to the sea is directly from ships, aquaculture pens, moorings, and industrial cooling pipes to which products containing it have been applied. It may also enter the sea in runoff from agricultural areas, from boat repair yards, and through municipal wastewater and sewage sludge. TBT is found to provide effective protection for boat hulls at release rates < 4 mg/cm^2 day and has been a popular antifoulant because it maintains its efficacy for up to 5 years, compared to about 3 years for other conventional applications. Once released to the water, TBT is degraded by sequential debutylation to dibutyltin (DBT), monobutyltin (MBT), and eventually to relatively nontoxic inorganic tin compounds. The degradation time in water is short, with half-lives reported from days to a few weeks (Stewart and de Mora 1990; Dowson et al. 1993). TBT is strongly particle-reactive, with partition coefficients reported to be as high as 10^3 to 10^4 (Langston and Pope 1995). The breakdown of TBT in anaerobic sediments is much slower than that in water (Clark EA et al. 1988). Therefore, contaminated sediments are potentially an important environmental reservoir for TBT that can continue to provide a source long after the industrial use of TBT has been curtailed. TBT is moderately lipophilic and will, therefore, bioaccumulate in the marine environment.

Surfactants

Alkyl phenol ethoxylates (APEs): These surfactants (usually nonylphenol ethoxylate or octylphenol ethoxylate) are used in industrial detergents, such as those used for wool washing and metal finishing; domestic detergents, such as clothes washing liquids; some shampoos, shaving foams, and other cosmetics; laboratory detergents, including Triton X-100; and pesticide formulations. APEs are being phased out by the European Union countries to be replaced by alcohol ethyoxylates, but in North America APEs continue to be used, especially in liquid detergents.

When alkyl phenol ethoxylates break down in sewage treatment or a river, they produce 3 main groups of alkyl phenolic compounds: alkyl phenol ethoxylates with fewer ethoxylate groups, alkyl phenoxy carboxylic acids, and alkyl phenols. Studies

in Switzerland have shown that these compounds persist in rivers and their sediments and in groundwater (e.g., Ahel et al. 1994, 1996). Nonylphenol di-ethoxylate (NP2EO) was found to be the most persistent degradation product (Ahel et al. 1994). Concentrations of NP2EO ranged from 2 to 8 mg/L in the River Glatt in Switzerland (Ahel et al. 1994) downstream of Zurich. Nonylphenol ethoxycarboxylate, the carboxylate analog of NP2EO, ranged in concentration from < 0.4 to 11.8 mg/L in the Fox River in Wisconsin, with highest concentrations downstream of pulp mills and municipal sewage treatment plants (Field and Reed 1996).

Alkyl phenols: Alkyl phenols (usually nonylphenol or octylphenol) are industrial products as well as degradation products of APEs. Alkyl phenols are used as antioxidants in some clear plastics, to prevent yellowing, in the form of tris-nonylphenol phosphite. They also are formed by degradation of triaryl phosphate lubricants (e.g., t-butylphenol, diphenyl phosphate) (Muir 1984). Concentrations of nonylphenol in receiving waters in the River Glatt in Switzerland, an industrial area, were found to be 2 to 4 mg/L (Ahel et al. 1994). Similar concentrations of nonylphenol (NP) have been reported in U.S. rivers (Naylor et al. 1992). Nonylphenol was rapidly accumulated from water by rainbow trout and eliminated with a half-life of 19 h (Lewis and Lech 1996). McLeese et al. (1981) determined a half-life of 4 d in salmon. They also found that depuration half-lives of alkyl phenols varied with chain branching and length. Longest half-lives in salmon were found for dodecyl phenol and shortest for p-sec-butylphenol. Analyses of fish from a river in Switzerland, near municipal and industrial sources of NP and nonylphenol ethoxylates (NP1EO), showed that NP, NP1EO and NP2EO were present in algae and fish. Highest concentrations of all 3 compounds were found in the gut, liver, and gill tissue; lowest concentrations were found in muscle. Bioconcentration factors (BCFs), (wet weight concentration in muscle/water) ranged from 50 to 100 for NP and from 5 to 250 for NP2EO (Ahel et al. 1993). McLeese et al. (1981) reported an equilibrium BCF of 280 for NP in salmon in flow-through experiments.

Modeling fate and transport and speciation of chemicals in the environment

Oviparous organisms include both aquatic and terrestrial representatives. Depending on the specific species and life stage, direct exposure to chemicals in primary compartments (i.e., air, water, soil, sediment) may occur through inhalation, ingestion, or dermal routes. Indirect exposure to concentrations in secondary compartments (i.e., plants, biota) may also occur via the dietary route. Table 2-2 illustrates the various environmental compartments and potential exposure routes to be considered. Maternal transfer to the egg represents a unique exposure pathway for oviparous organisms from development to hatching.

Table 2-2 Summary of potential exposure pathways

Exposure route	Environment compartment	Inhalation route	Ingestion route	Dermal route
Direct	Water (dissolved)	A	A*,T	A
	Water (particulate)	X	A,T	X
	Air (vapor)	A,T	X	X
	Air (aerosol)	A,T	X	X
	Soil (particulate)	X	T	T
	Sediment (particulate)	X	A	A
Indirect	Plant tissue	X	A,T	X
	Animal tissue	X	A,T	X

A = Aquatic, marine and estuarine species
T = Terrestrial species
X = Assumed to be insignificant pathway
*Ingestion of water may be a potential exposure route for andromanous species

Exposure to primary compartment concentrations

Primary compartment concentrations depend upon

- the nature of emissions, i.e., both the amount released and the release scenario
- the characteristics of the environment (e.g., advective flows, compartment sizes, and organic carbon content);
- the physicochemical properties of the contaminant that determine partitioning behavior within and between compartments; and
- the chemical/compartment-specific degradation half-lives (Mackay 1991). Emission estimates often are characterized poorly, although crude estimates for regional scale assessments may be obtained based on production volume, physicochemical properties, and use patterns of the chemical (van der Poel et al. 1995).

A generic "unit world" can be used to define environmental properties or, alternatively, site-specific information can be used if appropriate. The key physicochemical properties dictating the multimedia distribution of organic chemicals are the air-water (K_{aw}), octanol-water (K_{ow}), and octanol-air (K_{oa}) partition coefficients. Research over the last few decades has provided considerable experimental data and quantitative structure property relationships (QSPRs) for determining the K_{aw} and K_{ow} for many chemical classes. In contrast, only limited information is available on K_{oa}, although recent work for predicting this property is promising (Finizio et al. 1997). Abiotic degradation rates in air and water can be determined experimentally or estimated using QSPRs (Karickhoff et al. 1991; Kwok and Atkinson 1995; Lyman et al. 1995; Meylan and Howard 1995). While standardized protocols for determining biodegradation are available (OECD 1992) and a number of biodegradation QSPRs have been published (Degnen et al. 1993; Boethling et al. 1994; Klopman et al. 1995), a serious limitation in applying multimedia fate models is the difficulty in parameterizing compartment-specific biodegradation rates (Boethling et al. 1995;

Hales et al. 1996; Federle et al. 1997). The stochastic nature of the factors governing primary exposure concentrations is also significant. For example, the variation of emissions in time and space and the temperature dependence of partition coefficients and degradation half-lives are expected to cause variability in exposure concentrations.

Exposure to secondary compartment concentrations

A number of models have been used to predict concentrations in secondary compartments (i.e., biota) from measured or estimated primary compartment concentrations. These models range in complexity from simple QSPRs that estimate bioaccumulation based on physicochemical properties such as K_{ow} or vapor pressure (e.g., Mackay 1982; Garten and Trabalka 1983; Travis and Arms 1988; Bintein et al. 1993; McKone 1993; Tolls and McLachlan 1994; Kraaij and Connell 1997) to mechanistic models that integrate such factors as organism bioenergetics, life history, food-chain structure, and contaminant-specific toxicokinetic information (e.g., Thomann 1989; Clark T et al. 1988; Fordham and Reagan 1991; Thomann et al. 1992; Gobas 1993). A major drawback of these models is that most of them have been developed based on data from a very limited class of contaminants (e.g., poorly metabolized compounds). Consequently, these models cannot be extrapolated generically to many chemical classes. In order to avoid misapplication of these tools in exposure assessments, such limitations must be recognized (Tell and Parkerton 1997). Future work is needed to extend such model frameworks to other contaminant classes. This effort should include an explicit description of biotransformation processes in the model framework.

Use of modeling tools in exposure assessment

Despite the uncertainties and limitations of multimedia and bioaccumulation models, these tools provide important insights for assessing exposure to oviparous vertebrates. For new chemicals in which field measurements are not possible, this may be the only approach. In the case of existing chemicals, model results, if coupled with information on exposure factors (e.g., inhalation rates, ingestion rates, surface area for dermal uptake) for the receptor species of interest, can be used to prioritize which environmental exposure pathways are most important (i.e., what is the relative magnitude of the administered dose via different pathways?). This information can then be used to prioritize field monitoring efforts to characterize concentrations in the most critical environmental compartments. These calculations can also provide guidance in identifying the most relevant route of exposure to investigate in toxicity studies. For example, if the dominant pathway appears to be through the diet, priority should not be given to toxicity studies that examine exposure to the contaminant by air or water. An excellent compilation of exposure factors for wildlife that can be used for such calculations has been published by the USEPA (1993).

The above discussion provides a first step in understanding how the environmental fate properties of a contaminant influence potential exposure. However, if the ultimate objective is to link exposure estimates to a toxicological effect, the ability of the administered dose to be absorbed by the organism and then transported to the target site of action must be considered (Figure 2-1). While recognizing the important distinction between administered and absorbed dose, the extent to which such information can be considered may be constrained by the nature of the available toxicity information. If, for example, the only dose-response data that is available is

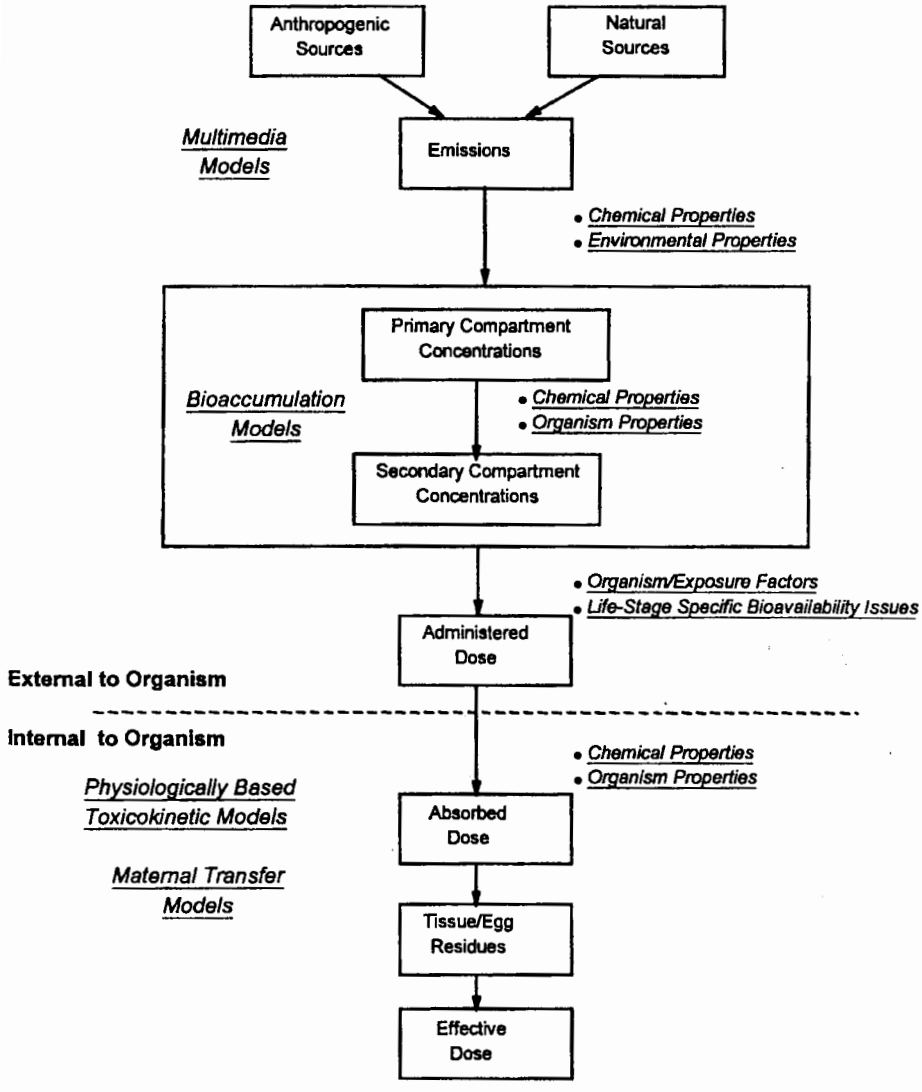


Figure 2-1 Key components influencing contaminant exposure

based on administered dose without additional information on tissue dosimetry it may not be possible to progress the exposure assessment beyond this point. Herein lies one of the main advantages of linking adverse effects of a contaminant to either whole body or specific target organ residues since concentrations in the organism provide an indirect measure of the absorbed dose. While a clear linkage between residue concentrations and reproductive or developmental effects has been shown for certain contaminants (e.g., DDT, organometalics, dioxin equivalents) such relationships may not exist for many other classes of contaminants particularly if they are readily metabolized. Conclusions reached in Ingersoll et al. (1997) also identified that the toxicological relevance of tissue residue data is an important research priority.

Quantifying external exposure

Defining bioavailability

The ability of a contaminant to be transported from the external environment to the target site within the organism depends upon: 1) the form (e.g., particulate, complexed, or freely dissolved) and chemical properties of the contaminant; 2) the characteristics of the organism that influences the efficiency by which the contaminant is extracted from the external environment (e.g., surface areas of respiratory, skin, and gut; ventilation and ingestion rates; enzymatic activities) via a given exposure route; and 3) the toxicokinetic behavior of the contaminant within the organism that determines the fraction of the absorbed dose that actually reaches the target site (i.e., the effective dose). Differences in contaminant toxicodynamics between species often form the mechanistic basis for explaining differences in toxicological sensitivity and thus extrapolation between species (Lawrence and Gobas 1997).

Confusion in the use of the term "bioavailability" arises as a result of differences in opinion regarding which of the above elements should be included in the definition (Dickson et al. 1994; Peijenburg et al. 1997). From an environmental chemist's perspective, the term bioavailability is usually limited to chemical speciation external to the organism. For example, truly dissolved concentrations of a nonpolar organic chemical are assumed to be bioavailable, while contaminant bound to dissolved organic carbon is not bioavailable (Landrum et al. 1985; McCarthy and Jiminez 1985; Black and McCarthy 1988). Thus, according to this view, the bioavailability of a chemical is independent of the organism. In contrast, ecotoxicologists and pharmacologists usually define bioavailability in terms of an absorbed dose so that the effect of the contaminant on the organism becomes implicit in the definition. For example, if the absorption efficiency of a chemical is 80% for a clam but only 60% for a trout, according to the latter definition, bioavailability in the clam is higher than in the trout.

Since the former definition limits bioavailability to chemical speciation, this restriction is problematic when applied to dietary contaminants. For example, hydrophobic organic contaminants will be primarily associated with the lipids in food and organic carbon in soils and sediments. Because the external chemical speciation is similar, the bioavailability of contaminants from different diets and soils/sediments is expected to be similar. However, experimental evidence contradicts this simple view (Vetter 1983; Landrum et al. 1992; Parkerton 1993; Alexander 1995). If instead bioavailability is defined in terms of an absorbed dose, a quantitative estimate of bioavailability can, in principle, be experimentally determined for different diets or soils/sediments. Moreover, differences in the bioavailability of various exposure routes can be directly compared.

Approaches for assessing bioavailability

Previous research indicates that observed toxicity to aquatic organisms correlates to the freely dissolved rather than total chemical concentrations. As a result, analytical methods or predictive models that enable freely dissolved concentrations to be estimated often have been used as a first step in accounting for contaminant bioavailability. Three commonly used techniques to determine freely dissolved concentrations are gas-sparging, equilibrium dialysis with semipermeable membrane devices (SPMDs), and filtration in conjunction with solid-phase extraction (Gustafson and Dickhut 1997). Equilibrium partitioning models have been widely used to account for the differences in bioavailability of sediment and soil contaminants (DiToro et al. 1991; van Leeuwen et al. 1992; Belfroid 1996; Ankley et al. 1996). While this approach is pragmatic, such models may not accurately represent bioavailability in the field (Ronday et al. 1997). Numerous studies examining contaminant desorption from sediments/soils indicate a biphasic behavior that consists of an easily desorbable and more resistant fraction (Pignatello and Xing 1995). The relationship between contaminant sequestration in soils/sediments and bioavailability has enormous implications for regulatory and remediation decisions and is consequently a major focus of current research (McGroddy et al. 1996; Loehr and Webster 1996; Kelsey and Alexander 1997; Gustafsson et al. 1997; Tomson and Pignatello 1997). In addition to sequestration mechanisms, other factors may limit the applicability of simple equilibrium partitioning theory (Belfroid et al. 1996; Peijenburg et al. 1997).

To provide a more accurate characterization of contaminant bioavailability, a variety of experimental approaches have been proposed. One commonly used technique is the use of a passive sampling device (SPMD containing lipid, solid-phase sorbent) that acts as a surrogate for organism lipid (Huckins et al. 1993; Corrol et al. 1994; Verhaar et al. 1994; Parkerton and Stone 1996; Lake et al. 1996; van Loon et al. 1997). To better account for organism-specific factors that may influence contaminant bioavailability, bioaccumulation and/or toxicity tests may be used. Such tests have been used for determining bioavailability of both organics (Harkey et al. 1995; McFarland 1995; Boesa et al. 1997; Meier et al. 1997; White et al. 1997; Kane-

Driscoll and Landrum 1997) and metals (Ankley et al. 1996; Ruby et al. 1996; van Gestel and Van Diepen 1997). Bioassays may be more realistic than biomimetic extraction approaches, but have the drawback of being significantly more cost- and time-intensive.

Effect of animal life history and behavior on chemical exposure

Allometric relationships controlling relative rates of diffusive and dietary uptake

The 2 main routes of exposure are diffusive uptake across gill and skin surfaces and dietary exposure via active feeding. While the latter is driven by contaminant levels in prey and bioenergetic-based food consumption rates, the former depends largely upon the diffusional gradient between the chemical in the organism's circulatory system and in the surrounding fluid (dissolved in water for aquatic organisms and in the gas phase for terrestrial organisms). Provided that chemical delivery to the surface is not limiting delivery of chemical via diffusive uptake scales to the concentration gradient and to the interfacial surface area, but the resulting concentration of chemical within the organism is determined by normalizing the delivered load to the mass of organism. Thus, the organism's surface-area-to-volume ratio is a direct index of the potential importance of diffusive uptake as an exposure pathway.

The surface-area-to-volume ratio of organisms that maintain reasonably constant shape during growth (i.e., finfish) decreases nearly exponentially, with dramatic decreases occurring in the period between egg hatching and growth to juvenile. Coupled with the lack of feeding during the early stages of larval development, this simple allometric relationship implies that diffusive uptake is likely to be the dominant exposure pathway to organisms with small embryonic and larval stages. Therefore, exposure of dissolved chemical via diffusive uptake may be an important route of exposure to sensitive life stages, even for those species whose adults are exposed primarily through their diets. This analysis cannot be extrapolated to those organisms, such as birds, who experience their exponential growth within the confines of an egg.

Changes in diet as animal matures

Animals may experience variations in dietary exposure of contaminants during their lifetimes because of shifts in their diets. Most fish begin with primarily planktonic or detrital diets and progress to larger prey items as they grow. As both contaminant levels and nutritional value of these various foods differ, exposure via diet varies. For example, concentrations of PCBs and polycyclic aromatic hydrocarbons (PAHs) are more than 10-fold enriched in macro-zooplankton relative to those of smaller plankton in the Chesapeake Bay (Ko and Baker 1995). Changes in diet with development can alter xenobiotic exposure. For example, white perch shift from a primarily phytoplanktonic diet to a mixed diet of zooplankton and benthos after 2 years of age. This shift has been shown to alter exposure pathways of Kepone to white perch age classes in the James River (Connolly and Tonnelli 1985). Kepone levels in

phytoplankton reflect dissolved Kepone levels in the surface waters, which declined rapidly after discharge of Kepone to the river stopped. Dietary exposure of Kepone to young herbivorous white perch declined as well. In contrast, older white perch eating a mixed diet of zooplankton and benthos (primarily the polychaete *Nereis*) maintained higher levels. Presumably Kepone was concentrated in zooplankton and mobilized from the sediments through ingested benthos.

Varying exposure due to migration

Mobile animals may experience varying exposure as they move among areas with varying contaminant levels. Movements include both local meanderings on daily time scales and long-distance migrations. Recurring seasonal migrations, such as those of migratory waterfowl and anadromous fishes and single migrations to spawn by species such as salmon, may drive temporally varying exposure. Assessing exposure and the resulting internal disposition of the chemicals is complicated by the fact that migrating organisms are also generally undergoing major physiological stress during migrations, resulting in changes in lipid reservoirs and bioenergetics.

As an example of seasonal migration as a control on exposure variability in an anadromous fish population, Zlokovitz and Secor (1999) have recently employed otolith microchemistry techniques to relate PCB levels in Hudson River striped bass (*Morone saxatilis*) to their migration patterns. Using energy-dispersive, x-ray spectroscopy coupled with scanning electron microscopy, the strontium/calcium ratios of individual otolith annuli can be measured (Secor 1992). Based on laboratory-derived calibration curves between salinity and Sr/Ca rations, the lifetime habitat salinity and migration patterns of each individual can be recreated. Figure 2-2 shows the migration history of a 15-year-old female striped bass collected from the Hudson River. After a 4- to 5-year period of irregular movement in the mesohaline reaches of the river, this individual female undertook regular annual migrations from the coastal ocean into less saline water. Other individual striped bass undergo dramatic shifts in their selected habitat, quickly moving from the river to the coastal waters or vice versa. The 7-year-old male striped bass in Figure 2-3 spent the first half of its life in high salinity water before moving into the upper reaches of the tidal Hudson. Although this fish contains fairly high levels of PCBs in its tissue (10.4 mg/g wet weight), it seems likely that this level reflects exposure during the second half of its life. In contrast, the other male striped bass shown in Figure 2-3 lived in low salinity waters under elevated PCB exposure before moving into saline waters after 2 years. Although levels of PCBs in its tissue at the time of capture were relatively low (0.4 mg/g wet weight), the reconstructed salinity history suggest that this fish was exposed to higher levels of PCBs during its early life. Movement into and out of contaminated areas by parental stock or during early developmental stages may very well determine the success or apparent impact of xenobiotics upon local populations.

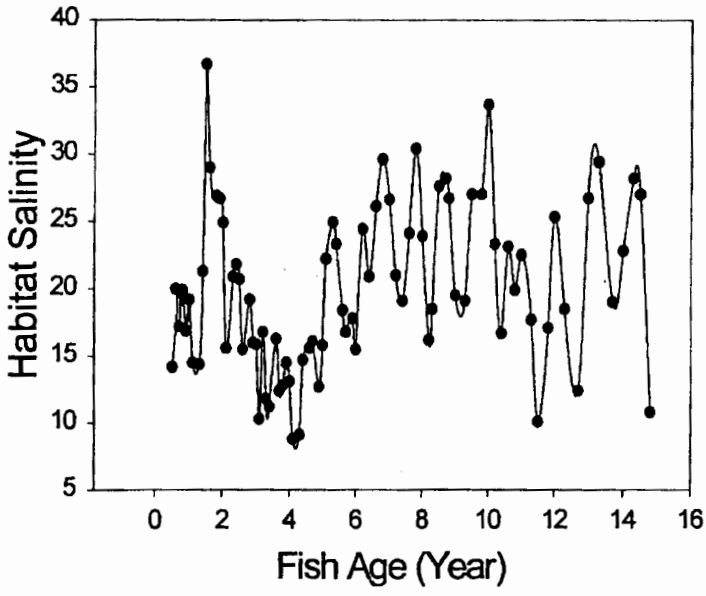


Figure 2-2 Reconstructed migration history of 15-year-old female Hudson River striped bass (Zlokovitz and Secor 1999)

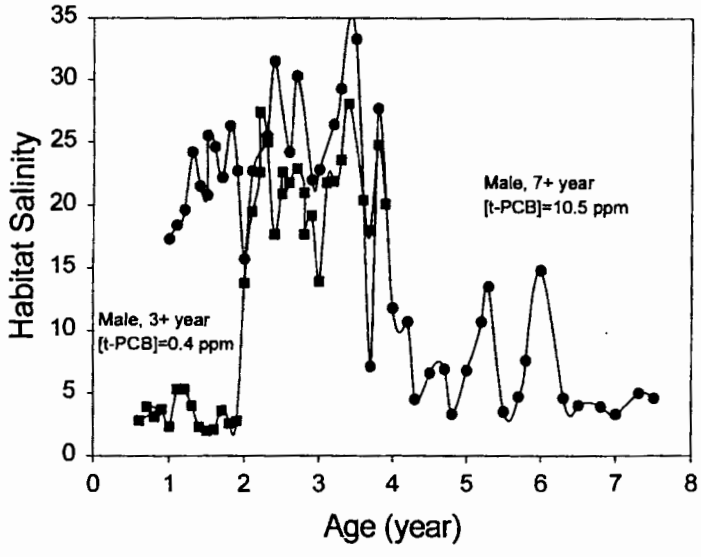


Figure 2-3 Contrasting migration behaviors of 2 male striped bass collected in the Hudson River. Total PCB concentrations reflect elevated exposure in freshwater regions of the river (Zlokovitz and Secor 1999).

Food-web structure and trophic transfer

Productivity and bioenergetics

Several recent studies have demonstrated that organisms living in more productive (e.g., more eutrophic) systems are exposed to lower levels of contaminants. In a study of 61 lakes in southern Scandinavia by Larsson et al. (1992), it was determined that levels of persistent pollutants (e.g., PCBs and DDT) in northern pike decreased as lake productivity increased, despite similar external pollutant loadings. They attributed this relationship either to the higher growth rate of the fish or to the decreased exposure resulting from enhanced sedimentation and complexation of the chemicals in the more eutrophic lakes. Using a model planktonic ecosystem, Millard et al. (1993) demonstrated that a larger fraction of added PCBs was bound by settling particles and colloids in more productive mesocosms. This model also showed that exposure to added PCBs decreased more rapidly in highly mixed, low-productivity treatments because of enhanced volatilization. In laboratory culture studies, Sijm et al. (1995) found that the bioconcentration of hydrophobic organic chemicals (HOCs) by 2 species of algae was inversely dependent upon algal density. They demonstrated an algal density-dependent production of organic exudates that bind HOCs and lower exposure levels. Paradoxically, evidence now suggests that relatively remote, oligotrophic water bodies with low external contaminant loadings (i.e., northern Great Lakes, Arctic Ocean) support elevated levels of contaminants in higher trophic levels (Tanabe et al. 1983; Muir et al. 1988; Norstrom et al. 1988; Swackhamer and Hites 1988; Hesselberg et al. 1990; Evans et al. 1991). These elevated tissue levels in water bodies with relatively low contaminant levels imply extremely efficient trophic transfer of particle-reactive chemicals in these oligotrophic systems.

Growth of aquatic organisms dilutes chemical concentrations within tissues even without net chemical exchange with the surrounding water (Thomann 1981). Swackhamer and Skoglund (1993) suggest that rapidly growing plankton fail to reach sorptive equilibrium with dissolved HOCs in the surrounding water due to the continual production of sorptive phase (i.e., biomass). This sustained disequilibrium maintains diffusional gradients and likely "pumps" dissolved contaminants into the base of the pelagic food web at a rate proportional to the algal growth rate. In these studies, algal growth rates were determined by temperature, both in the laboratory (Swackhamer and Skoglund 1993) and in the field (Swackhamer and Skoglund 1991). Contaminant exposure to secondary producers and predators, both through diffusive exposure across gill surfaces and through dietary exposure, likely scales to the organism's metabolic activity (Thomann 1981). Bioenergetic models have been used to explore the functional relationship between energetic requirements (i.e., prey consumption and gill ventilation rates), feeding strategy (i.e., food preference), and contaminant exposures (e.g., Thomann et al. 1992; Madenjian et al. 1993). These relationships are commonly studied in isolation on individual fishes in short-term laboratory studies (e.g., Gobas and Mackay 1987), which lack the

feedback between exposure levels, predator-prey interactions, and physiological functioning. Mesocosm-level studies, with adequate control and characterization of these feedbacks, will greatly improve our understanding of the interplay between bioenergetics and contaminant exposure.

Food-web structure

Recent studies have suggested that changes in trophic complexity (i.e., length of food chain) strongly impact dietary exposures of persistent chemicals to higher trophic levels. In a survey of lakes in Ontario, Rasmussen et al. (1990) found that concentrations of PCBs in the tissue of top predators increased significantly as the length of the food chain increased. Lake trout collected in lakes containing *Mysis* and pelagic forage fish (smelt, ciscoes, alewife, whitefish) contained significantly higher lipid-normalized levels of PCBs than those taken from lakes lacking these intermediate trophic levels. Cabana and Rasmussen (1994) report a strong positive correlation between mercury levels and trophic position (as determined by $d^{15}N$) in biota from Ontario lakes.

Benthic-pelagic coupling may play an important role in enhancing exposure of aquatic organisms to particle-reactive chemicals. Epibenthic fishes common to coastal areas are exposed to contaminants both from water column sources and from transfer of sediment-associated contaminants through the benthic food web. Kucklick and Baker (1998) and Thomann et al. (1992) suggest that sediment-associated contaminants are responsible for the relatively slow response times of contaminant levels in higher trophic levels in the Great Lakes and elsewhere. While sediments are recognized as the "geochemical memory" of aquatic systems, the mechanisms and extent of bioavailability of historically contaminated sediments are open questions (e.g., Landrum et al. 1992; McGroddy et al. 1996).

Chemical Disposition in Oviparous Vertebrates as Related to Reproduction and Development

Chemical uptake and disposition in early life stages of fish

Overview

In the 1940s the lake trout (*Salvelinus namaycush*) in the Great Lakes demonstrated large population declines due to predation by the sea lamprey, overfishing, and loss of prime spawning habitat. Resolution of such issues and extensive restocking failed to foster natural reproduction. Subsequent studies established that the lack of reproductive success was related to the failure to produce viable offspring (Jude et al. 1981; Nester and Poe 1984; Marsden et al. 1988). Numerous studies documented the bioaccumulation of halogenated aromatic hydrocarbons in the Great Lakes lake trout (Stalling et al. 1983; Schmitt et al. 1985; Huckins et al. 1988; Niimi and Oliver 1989; DeVault et al. 1989) as additional investigations lead to correlations between

environmental contamination and mortality of early life stages (Willford et al. 1981; Mac et al.1985, 1988). Burdick et al. (1964), Mac et al. (1985), Spitsbergen et al. (1991), and Walker, Spitsbergen et al. (1991) provided evidence that concentrations of organochlorine compounds in lake trout eggs were inversely related to hatching success or fry survival. Xenobiotics transferred from the parental stock to the gametes were suggested as a reason for the declines noted. Contaminant concentrations found in eggs now have been correlated with reduced hatchability or survival in a number of fish species, including Atlantic salmon (*Salmo salar*) (Jensen et al. 1970), rainbow trout (*Oncorhychus mykiss*) (Hogan and Brauhn 1975), dace fry (*Phoxinus phoxinus*) (Bengtsson 1980), Arctic char (*Salvelinus alpinus*) (Monod 1985), white perch (*Morone americana*) (Monosson 1992), and chinook salmon (*Oncorhynchus tshawytscha*) (Ankley et al. 1991).

Numerous studies have demonstrated the toxicity of contaminants to the early life stages of fish. As compared to adults, developmental stages often appear to be more sensitive to the toxicity of xenobiotics (McKim et al. 1975; McKim 1977; Macek and Sleight 1977; Eaton et al. 1978; Sauter et al. 1976; Pickering and Thatcher 1970; Pickering and Gast 1972). Contaminant uptake, accumulation, and disposition are important modulators of toxicity in early life stages, as in the adult. Uptake of xenobiotic chemicals may occur at any stage of the fish life cycle. Early life stages may be exposed to xenobiotics as a result of maternal transfer to the egg prior to parturition or by direct environmental exposure post-parturition. Varying maternal contributions to the early life stage are presented throughout development as xenobiotics stored in the yolk and associated structures are mobilized and depleted. Direct exposure for the egg occurs primarily via water/chorion exchange. Following hatch, the yolk-sac, larval stage may be directly exposed through dermal and branchial routes. With consumption of the yolk, post-yolk-sac larvae present a new dosing paradigm, as, in addition to dermal and branchial exposure, the animal is now feeding. Similar to absorption, possible elimination routes vary with the stage of development. Xenobiotic elimination from the egg or embryo is a multiphasic event including loss from the embryo proper into the surrounding structures and fluids of the egg and from the totality of the egg across the chorion (or comparable membrane) to the environment. Development from the egg to the yolk-sac larvae (or equivalent stage) alters the complement of elimination routes with the advent of a functional circulatory system in conjunction with newly functional kidneys and gills. Branchial and renal routes of elimination in combination with dermal processes are likely to play an important role. Past the yolk-sac stage (swim-up), the biliary and gastrointestinal tracts become functional, again altering the complement of routes of xenobiotic elimination. Interposed upon the development of these elimination routes is the ontogeny of biotransformation enzymes and their ensuing activity.

This section reviews chemical uptake and disposition in early life stages of fish. A variety of excellent reviews are available regarding these issues in adult fishes for the

reader to peruse and contrast. In the current discussion, the adult is included only for those topics in which they are an integral part of the process, such as with maternal transfer. This discussion follows the order of exposure and perceived importance: maternal then direct exposure. The stage will be set by discussing the scope of the contaminant problem in early life stages and the relationship with adult body burdens. This will be followed by a more in depth discussion of maternal transfer of xenobiotics. Factors influencing maternal transfer and the processes involved will be addressed in this section. Direct uptake and disposition of chemicals in early life stages will follow. Routes and patterns of absorption, elimination, and biotransformation in early life stages will be emphasized.

Xenobiotic residues in gametes of fish

A variety of contaminants have been found in the eggs of wild fish with the implication of potential effects upon reproduction and development. Most of the studies examining contaminant concentrations in eggs have focused on persistent organochlorine compounds, although some work has been carried out on metals or metaloids such as selenium, mercury, lead, copper, and cadmium. A fairly diverse group of fish species has exhibited xenobiotic residues in eggs; however, the predominance of data resides with salmonids. Studies as early as the 1960s have identified DDT and PCB residues in eggs of salmonid species (Willford et al. 1969). Studies by Miller (1993) and Miller and Amrhein (1995) have provided a more comprehensive examination of a wider variety of organochlorine residues in eggs from chinook salmon (*Oncorhynchus tshawytscha*), lake trout (*Salvelinus namaycush*) and siscowet (*Salvelinus namaycush siscowet*). PCBs, toxaphene, *p,p'* DDE, *o,p'* DDE, *p,p'*DDT, and *p,p'*DDD have been identified in eggs from Lake Michigan chinook salmon (Miller 1993). Similarly, Lake Michigan lake trout eggs were shown to contain PCBs, toxaphene, *p,p'*DDE, *p,p'*DDT, and *p,p'*DDD. Lake Superior lake trout (siscowet), while demonstrating a profile similar to their Lake Michigan counterparts, exhibited overall lower xenobiotic concentrations. Egg concentrations of these compounds (collected in 1982) ranged from 0.3 to 8.3, 1.0 to 2.6, 0.15 to 2.3, 0.08 to 0.14, and below detectable limits to 0.15 ppm for PCBs, toxaphene *p,p'*DDE, *p,p'*DDT, and *p,p'*DDD, respectively. Eggs collected in 1991 from Lake Superior lake trout, also have demonstrated detectable concentrations of PCBs (0.45 ppm), transnonchlordane (0.03 ppm), *p,p'*DDE (0.09 ppm), and dieldrin (0.02 ppm) (Miller and Amrhein 1995). With the exception of PCBs, concentrations of like compounds in siscowet were generally lower than their 1982 lake trout counterparts (Miller 1993; Miller and Amrhein 1995). Eggs from Lake Ontario rainbow trout contained PCB, *p,p'*DDE, ΣDDT, α chlordane, Σchlordane, mirex, heptachlor epoxide, dieldrin, hexachlorobenzene (HCB), and mercury at concentrations of 2050 ± 700, 293 ± 97, 458 ± 149, 61 ± 18, 76 ± 25, 71 ± 29, 2 ± 1, 20 ± 9, 14 ± 7, and 11 ± 8 mg/kg, respectively (Niimi 1983). Heavy metals and organochlorine compounds also have been detected in coho salmon (*Oncorhynchus kisutch walbaum*) eggs collected from Lakes Michigan, Erie, and Ontario (Morrison et al. 1985). PCB, *p,p'*TDE, *o,p'*DDT, dield-

rin, *p,p'*DDE, chlordane, zinc, lead, mercury, copper, and cadmium were found in the eggs from all 3 sources. Additional studies have expanded the list of detected compounds to include polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), which were identified in eggs from lake trout (Walker et al. 1992).

Species of fish other than salmonids have also been shown to exhibit gonadal accumulation of contaminants. Eggs collected from winter flounder (*Pseudopleuronectes americanus*) (New Bedford Harbor, MA) demonstrated significantly higher levels of PCBs (39.6 mg/g dry weight) than at a control site (Fox Island: 1.08 mg PCB/g dry weight) (Black et al. 1988). Studies with Baltic flounder (*Platichthys flesus*) demonstrated significant ovarian tissue residues of a variety of contaminants including PCBs, hexachlorocyclohexane (Lindane -αHCH), γHCH, DDD, dieldrin, HCB, heptachlorepoxide, and zinc (Von Westernhagen et al. 1981). Residue levels in the ovaries from these fish ranged from 5.0 to 317.0, 0.7 to 6.0, 0.4 to 5.6, 3.0 to 30.0, 0.1 to 49.0, 0.6 to 2.0, 0.08 to 3.0 ng/g wet weight, and 3.7 to 31.7 mg/g for the foregoing contaminants, respectively. Eggs collected from Hudson River striped bass (*Morone saxatilis*) also exhibited PCB concentrations ranging from 1.1 to 8.1 mg/g wet weight (Westin et al. 1983). An interesting feature of these data was that the highest PCB concentrations in the eggs came from the smallest female, while the lowest concentration was associated with the largest animal. Paddelfish (*Polyodon spathula*) have also been shown to accumulate significant levels of PCBs in both ovaries and testis (Gundersen and Pearson 1992). Aroclor 1260 concentrations ranged from 0.05 to 18.70 (mean = 7.3) and 5.63 to 23.00 (mean = 16.2) mg/g for ovaries and testes, respectively. Interestingly, PCB levels in mature roe of paddlefish were lower than that of immature ovaries. The authors attributed this finding to a change in lipid content. Mature ovaries, while much larger, had considerably lower lipid content than their immature counterparts. Niimi (1983) examined the deposition of a variety of contaminants in the eggs of various fish species collected from Lakes Ontario and Erie, including white sucker (*Catostomus commersoni*), white bass (*Morone chrysops*), smallmouth bass (*Micropterus dolomieui*), yellow perch (*Perca flavescens*), and rainbow trout (*Salmo gairdneri*). Residues of PCBs, *p,p'*-DDE, DDT, α chlordane, Σchlordane, heptachlor, dieldrin, HCB, and mercury were found in the eggs of all species. Smallmouth bass eggs maintained the highest residue levels on a mg/kg basis for 5 of the 9 chemicals analyzed in all species. Conversely, yellow perch contained the lowest levels for 7 of the 9 chemicals. Not surprisingly, given the nature of the compounds, this general trend correlated to the percentage of lipids in the eggs of these 2 species.

A considerable amount of work has focused upon the reproductive and developmental toxicity of selenium. Hence, a number of studies have focused upon selenium content in the gametes of fish from impacted waters. Investigations by Gillespie and Baumann (1986) focused upon selenium concentrations and reproductive and developmental performance of bluegills collected from Hyco Reservoir (Roxboro,

NC), a selenium-contaminated environment, and Roxboro City Lake as a reference site. Selenium concentrations in the ovaries and testes mirrored carcass and environmental exposure. For the control site, mean selenium concentrations were 0.37 and 0.50 mg/kg for carcass and testis, respectively for males, and 0.37 and 0.66 mg/kg for the carcass and ovaries of females. There was a similar comparison for Hyco reservoir animals: males had concentrations of 7.81 and 4.37 mg/kg for carcass and testis, respectively, and females had concentrations of 5.91 and 6.96 mg/kg for carcass and ovaries. These results suggest that, while perhaps not preferentially accumulating in gametes, selenium does distribute to gametes as a reflection of exposure. A similar finding was evident for selenium in eggs and milt of the razorback sucker (*Xyrauchen texanus*) (Hamilton and Waddell 1994).

Clearly, the existing literature identifies a wide range of fish species that contain contaminants in their eggs. It is apparent that for numerous fish species, persistent lipophilic organochlorine compounds can be passed along from the parent to their gametes. Although not as extensively documented, it has been demonstrated that a number of metals or metaloids are forwarded to gametes in natural settings. Studies with less persistent compounds have not been reported with regard to parental transport to the gametes.

Correlation of maternal nonreproductive tissue residues to egg or ovarian contaminant levels

Correlations have been made between egg or ovarian contaminant levels and maternal nonreproductive tissue residues, lending insight into xenobiotic partitioning and providing a potential tool for predictive assessments. Total concentrations of PCBs, *p,p'*DDE, and dieldrin in lake trout eggs and total concentrations of PCBs and *p,p'* DDE concentrations in chinook salmon eggs have been significantly correlated with concentrations of these chemicals in the muscle tissue of gravid fish (Miller 1993). While this is true, the relationship between muscle and egg organochlorine concentrations differs between the two species. Higher contaminant concentrations were found in chinook salmon eggs relative to their muscle tissue, whereas in lake trout higher contaminant levels were found in the muscle tissue relative to the eggs (Miller 1993). Correlations between organochlorine concentrations in the muscle tissue and eggs were greatest for compounds found at higher concentrations. In addition, the correlation coefficients for the relationships between muscle and egg organochlorine concentrations were higher for the lake trout than for chinook salmon (R = 0.62 to 0.67 for chinook salmon versus 0.94 to 0.96 for lake trout). The author suggested that reproductive staging of collected individuals may have a strong effect on observed residues in both the eggs and muscle of chinook salmon as both loss of appetite prior to spawning and somatic lipid mobilization for egg development are pronounced components of the chinook life history. In a similar study, with siscowet lake trout, concentrations of PCBs and *p,p'*DDE in eggs were also positively correlated with muscle concentrations (Miller and Amrhein 1995). In contrast, cis-chlordane, cis-nonchlordane, and *p,p'* DDT

evident in muscle at concentrations ranging from 0.06 to 0.14 mg/kg were undetected in eggs (0.05mg/kg detection limits). For those compounds detectable in both muscle tissue and eggs, concentrations in muscle ranged from 5 to 8 times higher than concentrations in eggs. Again, like the Lake Michigan lake trout, organochlorine concentrations were significantly higher in the muscle than in the eggs of the siscowet. TCDD concentrations in muscle of maternal lake trout also appear to correlate to TCDD concentrations in their eggs (Walker et al. 1994). The TCDD concentration in eggs were 42 and 43% of skeletal muscle levels when expressed on a lipid and wet-weight basis. This compares to values for total PCB concentrations in lake trout eggs of 22 to 38% of the total PCB in maternal skeletal muscle on a wet weight basis and 66 to 78% on a lipid-normalized basis (Miller 1993). Similar residue comparisons have been made among muscle, liver, and ovaries of Northwest Atlantic cod (Hellou et al. 1993). Generally, concentrations of 23 specific organochlorine contaminants were undetectable in cod muscle tissue, while the ovaries presented detectable concentrations that were 10 times lower than liver tissue on a wet-weight basis (Hellou et al. 1993). A number of compounds including αHCH, β-HCH , γHCH, and oxychlordane were shown to have substantial negative correlations between concentrations in the ovaries and the liver. Cis/trans chlordane, trans-nonachlor, cis-nonachlor, *p,p'*DDE, *p,p'*DDT, Aroclor1254 (weak), *p,p'* DDD, and *o,p'* DDD generally exhibited positive, or at least non-negative, correlations between concentrations in liver and ovaries in cod. In studies with paddlefish, PCB concentrations were much higher in reproductive tissues than in corresponding muscle tissues (Gundersen and Pearson 1992). Average ovarian PCB concentrations were 1.7 to 18.2-fold higher than those of muscle, dependent on whether the comparison was made with white muscle (1.7) or red muscle (18.2). Male gonadal tissue concentrations were 23-fold higher than were white muscle concentrations. Other studies examining PCB concentrations in winter flounder from New Bedford Harbor, MA, showed that the content of PCB congeners in the liver is influenced little by sex or reproductive condition (Elskus et al. 1994). Compositely, these studies suggest that the relationship of somatic or visceral xenobiotic residue levels to that deposited in the ovaries is highly species dependent.

Studies by Kammann et al. (1993) and Knickmeyer and Steinhart (1989) suggest that organ residues may vary with the spawning season. For example, at the beginning of the dab (*Limanda limanda*) spawning period, during high egg production, PCB and HCB concentrations are low in female livers, while the levels of these compounds increased during the same interval in the ovaries (Kammann et al. 1993). Maximal PCB and HCB burdens in dab ovaries were observed at the end of the spawning period in April. Studies with wild-caught dab suggest that PCB patterns in liver and ovaries were dominated by penta- and hexachlorobiphenyls with a large contribution from PCB 138 and 153 (Kammann et al. 1993). The PCB pattern in testes, on the other hand, was dominated by tri- and tetrachlorobiphenyls. PCB patterns of female dab livers collected in February and May were similar, with the exception of PCB 138. This congener was shown to be preferentially

transferred from dab liver to ovarian tissues during ovarian development, resulting in significant alterations in liver patterns (Knickmeyer and Steinhart 1989). PCB 28, 60, 66, and 99 were also present in higher concentrations in ovary than in liver at select samplings (February). Significant differences in congener profile occurred in female dab at the May sampling with greater representation by tri- and tetrachlorobiphenyls. The authors concluded that a change in lipids for ovary anabolism may have occurred. In winter months, when nutrition is poor, lipids transferred from the liver may be the main source for anabolism in ovaries leading to similar PCB patterns in these two organs. Increased feeding in spring resulted in the incorporation of the less-chlorinated compounds present in prey with ovarian maturation (Knust 1986). These conclusions will require further verification with additional studies.

Von Westernhagen et al. (1995) examined age- and length- dependent concentrations of chlorinated hydrocarbons in ovary and muscle of herring, flounder, dab, whiting, and horse mackerel. From these data, no generally acceptable pattern was recognizable between the development of tissue residues and age in sexually mature female fish (Von Westernhagen et al. 1995). The authors suggested that this may be because of gonadal maturation, whereby there is a transfer of lipids and associated xenobiotics from the liver to the gonads and a subsequent loss of gonadal materials upon spawning. It is thought that the seasonal release of eggs thus acts to remove excessive accumulation of contaminants.

Xenobiotic residues in gametes: laboratory studies

A number of experimental studies have demonstrated and quantitatively characterized transfer of chemicals to fish gametes. Hall and Oris (1991) demonstrated significant bioconcentration of anthracene in the eggs of fathead minnows (*Pimephales promelas*) exposed at 12 mg/L in the water. The fathead minnow BCFs noted for the male carcass was 1126, male testes 769, female carcass 3581, ovaries 1452, and eggs 759. Female yellow perch dosed with 2,5,2',5'-tetrachloro[^{14}C] biphenyl in the water demonstrated that 2 weeks after exposure, 30% of the initial biphenyl body burden was distributed to the eggs, whereas just prior to spawning, about 50% was present in this tissue (Vodicnik and Peterson 1985). In these studies eggs contained anywhere from 23.5 to 41.8 mg/kg of 2,5,2',5'-tetrachloro[^{14}C] biphenyl for the 18 weeks prior to spawning. Mean total ng PCB concentrations were much lower in perch fillet and viscera than in carcass and eggs during ovarian maturation. Total carcass PCB levels were high early in egg development (469 to 502 ng), but dropped precipitously (57 to 228 ng) as the egg PCB burden raised from 270 to 498 ng before spawning. This change could be accounted for in part by a large increase in egg mass. Additional laboratory studies with 2,5,2',5',-tetrachloro[^{14}C] biphenyl in rainbow trout demonstrate accumulation in maturing eggs and sperm following redistribution of residues from within the fish's body (Guiney et al. 1979). Following a 36-hour static, waterborne exposure to PCB, concentrations of radiolabelled PCB dropped in the whole fish, skin, carcass,

skeletal muscle, and liver. During this same interval, the percent of initial ^{14}C residue increased from 0.1 to 5% and from 0.1 to nearly 2% for eggs and sperm, respectively. Similarly, several experimental studies have demonstrated transfer of maternally derived TCDD to the eggs of lake trout (Walker et al. 1994). Female lake trout administered TCDD in the diet (total exposures 22.8 to 62.2 mg) for 11 weeks demonstrated widely varying TCDD concentrations for individual fish even within a treatment. Egg TCDD concentrations, which were generally lower than liver and muscle levels and higher than blood levels, ranged from 71 to 311 pg/g wet weight. Studies by Ungerer and Thomas (1996) examined the accumulation of *o,p'*-DDT and Aroclor 1254 in gonadal and hepatic tissues of the Atlantic croaker (*Micropogonias undulatus*) following dietary administration. For both compounds and sexes, the liver contained greater concentrations of the contaminants. For females the gonadal/liver contaminant ratios ranged from 0.645 to 0.794, while for males the ratios ranged from 0.009 to 0.037 for both compounds. Female gonadal tissue contained 118 and 38.6-fold higher levels than that of males on a mg/g basis for *o,p'*DDT and Aroclor 1254, respectively.

Factors influencing contaminant deposition in gametes

A number of studies have investigated the role of parental body composition upon contaminant disposition in the ovary. Niimi (1983) examined the relationship of lipid content of the fish, mean egg weight as a percent of the total fish weight, percent of total maternal lipid deposited in eggs, and percent of whole body contaminants in fish transferred to eggs. When the data were compiled for 5 fish species (rainbow trout, yellow perch, smallmouth bass, white bass, and white sucker), several interesting features emerged as related to a variety of organic contaminants, lipid content, and transfer efficiency. On a percentage basis, the yellow perch, although the leanest (5.1% lipid) species examined, transferred the greatest percentage of whole-body contaminants (25.5%). Rainbow trout, while having the highest lipid content (11.4%), had the lowest percentage of contaminants transferred to the eggs (5.5%). Interplaying on these 2 factors are mean egg weight as percent of total weight and percent of total lipid deposited in eggs. Contributing to the high percent of contaminants transferred to eggs in yellow perch was a relatively high mean egg weight as percent of total weight (yellow perch: 22.3 versus 13.6% for rainbow trout) and the high percent of total lipid transferred to eggs (yellow perch 27.1 versus 10.3% for rainbow trout). When all species are examined relative to total organic contaminant load in eggs on a mg/kg basis and also as related to the egg as percent lipid, the following comparison becomes available, given in order of highest to lowest composite contaminant concentration on a per kg basis (lipid percent in parentheses): smallmouth bass, 4080 mg/kg (13.4% lipid in eggs); rainbow trout, 2985 mg/kg (8.7%); white bass, 2771 mg/kg (9.7%); white sucker, 2501 mg/kg (5.7%); and yellow perch, 1078 mg/kg (5.8%). While it is clear that the composite concentration of these compounds in the eggs appear to be generally correlated to their lipid content, similarities in lipid content and disparities in residue levels

(perch and sucker) and similarities in residue levels and disparities in lipids (suckers and white bass) also appear. These results suggest that other factors may be influential modulators of residue loading in eggs on a mg/kg basis. It is worthy to point out that differences in egg concentrations of contaminants may be related to a variety of factors including species differences, contaminant levels in the environment, or even differences in gonadal development relative to contaminant deposition at the time of egg collection.

Comparative differences in the residues imparted to eggs are also evident between the lake trout and the chinook salmon (Miller 1993). In this case the reproductive life history also appears to play a determinant role in this process. Chinook salmon, semelparous (once-bearing) in nature, transfer most of their somatic lipid stores to developing gonads. The lake trout, an iteroparous (multiple-bearing) species, transfers a much lower proportion of their somatic lipids to the developing gonads (Miller 1993). This fact translates to estimates that gravid lake trout lose 3 to 5% of their total PCB body burden during spawning, whereas chinook salmon lose 28 to 39%.

There are numerous indications that different species have different life strategies for resource mobilization for ovarian development. Nassour and Leger (1989) suggested that carcass and visceral lipid reserves are mobilized for rainbow trout ovarian development. For the same purpose, freshwater catfish (*Clarias batrachus*) utilize abdominal fat (Lal and Singh 1987) and Atlantic salmon (*Salmo salar*) use muscle lipid and protein (Aksnes et al. 1986). In contrast, dietary lipid sources appear to be primary for ovarian development of the gilthead bream (*Sparus aurata*) (Harel et al. 1994) and the northern pike (*Esox lucius*) (Medford and MacKay 1978; Diana and MacKay 1979). Another feature that may come into play is the concept of differential distribution of contaminants in the parental stock. There are a couple indications in the literature beyond the foregoing discussion that differential distribution may play a role in contaminant deposition in gametes. It has been demonstrated that the disposition and elimination of organochlorine compounds may differ between fish species. Guiney and Peterson (1980) demonstrated that in rainbow trout, the major distribution sites for 2,5,2',5' [^{14}C]-tetrachlorobiphenyl were the skeletal muscle and carcass, accounting for 60% of the dose, while in yellow perch, 70% of the administered dose was contained in the viscera and carcass. This finding, as the authors suggest, may be related to species differences in the lipid content of the various tissues. Similar findings have been noted in other studies. Zitko et al. (1974) found that Atlantic herring (*Clupea harengus harengus*) had both a muscle PCB and a lipid content 5 to 10 times higher than those of yellow perch. While the direct source and the transfer dynamics of contaminants are unclear, such findings again suggest that life history and physiology may play a determinate role in the relative importance of maternal transport of lipophilic contaminants in a given species.

The influence of dietary energetics on the process of parental transfer of contaminants is largely unknown. Miller (1993) suggested that variations in chinook organochlorine concentrations in both muscle tissue and eggs may be related to energetic constraints resulting from forage limitations. Reduced size, lipid content, and growth rate have been noted for chinook salmon with such limitations. As compared to lake trout, chinook appear to have greater variability in somatic lipid stores which in turn may induce variability in lipids and contaminants transferred to the eggs. It has been shown that starvation-induced depletion of lipid stores in rainbow trout and coho salmon (*Oncorhynchus kisutch*) have failed to increase elimination of PCBs from the whole fish (Lieb et al. 1974; Gruger et al. 1975). It is plausible that if contaminants are not co-eliminated with lipid loss under conditions of nutrient limitations, then lipid mobilization to the ovaries under such conditions may increase the exposure potential to the eggs. Again, further investigations are necessary to examine the relative importance of these issues.

In totality, this information suggests that the deposition of contaminants in the egg mass may be directly linked with species-specific characteristics. Lipids and lipid dynamics are critical to lipophilic contaminant transfer to eggs. This transfer may be altered by increased or decreased lipid mobilization in response to the maternal lipid content and/or the reproductive life history of the animal. The toxicological significance of this transfer may center not only on the initial maternal concentration of contaminants, but also on the source and amount of the lipids transferred. High contaminant concentrations in maternal lipid coupled with low lipid transfer, such as with rainbow trout, may approach similar contaminant transfer conditions as those of low contaminant concentrations, such as in perch. The similarity in contaminant transfer with differences in contaminant load and lipid transfer may mean that tissue (muscle) concentrations of contaminants and contaminants in eggs may be related by some formulation on only a species-to-species basis.

Transport of contaminants to ovaries

Vitellogenesis and oogenesis in oviparous fishes

The onset of oogenesis initiates a host of physiological changes, including alterations in serum protein profiles, that may play an important role in the maternal transfer of endogenous and exogenous hormones, and xenobiotics may be transferred to oocytes. This alteration in serum lipoprotein (LP) content, a part of vitellogenesis, results from the dramatic, estrogen-induced synthesis of vitellogenin (VTG) and other lipoproteins. In oviparous fishes, the synthesis and sequestration of large quantities of LPs, particularly VTG, are critical elements of oocyte development. While catalytically processed and sequestered LPs serve primarily as a nutrient source for embryogenesis and early-life-stage development, the oocytic accumulation of some trace metals, micronutrients, and hormones also may be associated, in part, with oocytic accumulation of lipoprotein-associated ligands (Specker and Sullivan 1994). The oocytic sequestration of serum LPs, however, also

may serve as a vector of maternal transfer of xenobiotic compounds, facilitating embryonic and early-life-stage exposures.

Vitellogenesis and oogenesis in oviparous fishes, like all oviparous vertebrates, are endocrine-orchestrated responses to environmental stimuli such as changes in temperature, photoperiod, lunar cycle, and/or diet. During vitellogenesis, increases in serum estradiol (E2) levels lead to the production of a variety of lipoproteins, including the phospholipoglycoprotein VTG and very low-density lipoproteins (VLDLs). These proteins are taken into maturing oocytes by selective, receptor-mediated endocytosis. On a dry-weight basis, VTG is the major serum lipoprotein in vitellogenic females and forms the basis of yolk protein sequestered in maturing oocytes. In addition, while VLDLs typically contain greater molar quantities of lipid, the majority of lipid found in the oocyte of oviparous fishes is derived from proteolytic cleavage of VTG, not VLDL (Wallace 1985).

A great deal is known about oogenesis and the formation of oocytes in oviparous vertebrates, including the uptake of vitellogenin and other serum proteins. Detailed information regarding these processes is available in a number of seminal reviews, including those contributed by Chapman (1980), Wallace (1985), Byrne et al. (1989), Maller (1985), Wallace and Selman (1990), and Nagahama (1994). Likewise, a great deal of recent work continues to be done examining the effects of xenobiotic chemicals on early life stages of a number of oviparous vertebrate species including fish (e.g., Guiney et al. 1997; Olivieri and Cooper 1997; Fahraeus-Van Ree and Payne 1997), birds (e.g., Hoffman et al. 1987; Nosek et al. 1993; Stanley et al. 1994), amphibians (e.g., Bernardini et al. 1996; Dawson et al. 1996), and reptiles (Crews et al. 1996). Less is known, however, about the precise mechanisms of pollutant uptake by oocytes and the relative role LP incorporation plays in maternal transfer of xenobiotics.

While substantial similarities exist between oogenesis in different phyla of oviparous vertebrates, significant differences in protein biochemistry and life histories both within and between these phyla complicates overreaching generalizations. Critical questions addressed below, and in similar subsection for birds, focus on the role played by LPs in the transfer of xenobiotics from the female piscine to her maturing oocytes.

Elements of lipoprotein synthesis and transport

Vitellogenesis and oogenesis have been the focus of a great deal of research for over 40 years. Of central importance in the current discussion is the fact that most oviparous species, including fish, produce a variety of lipoproteins, typically differentiated in terms of their lipid density: VLDL, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and the VTG-containing very-high-density lipoproteins (VHDL). Of these, VTG and VLDL are the predominant LPs incorporated by maturing oocytes.

Once transported from the circulation into the oocyte, VTG is processed into smaller yolk proteins consisting of lipovitellins, phosvitins, phosvettes, and other cleavage products that represent the major source of nutrition during embryonic development and early life stages (Wallace and Selman 1990; LaFleur et al. 1995). Analysis of VTG-cleavage products from oocytes suggests that VTGs of oviparous fishes are frequently more lipidated and contain more widely divergent (though typically smaller) quantities of protein-phosphorous (Jared and Wallace 1968; de Vlaming et al. 1980; Craik 1982) than VTGs of other oviparous vertebrates.

Vitellogenin is perhaps the best studied oviparous lipoprotein and, while multiple forms of circulating VTG have been identified within specific species of fishes (Ding et al. 1989; Chan et al. 1991; Kishida and Specker 1993), birds (Wang and Williams 1980, 1983; Evans et al. 1988), and amphibians (Wiley and Wallace 1981, Wahli and Ryffel 1985), differences in VTG composition also exist between species within each of these phyla. Except where noted, however, vitellogenin and VLDL will be used as general terms inclusive of all forms of the proteins in a specific phyla (i.e., VTGs of all oviparous fishes will be considered to possess similar physicochemical composition and properties). During the discussion that follows, it should be kept in mind that intra- and interspecies differences in lipoprotein composition and oocytic uptake may result in differences in rates and magnitudes of maternal transfer. Future studies that improve our understanding of the structure and binding dynamics of lipoproteins from a greater diversity of oviparous fishes will greatly improve our ability to elucidate the role lipoprotein sequestration plays in maternal transfer of ligands to maturing oocytes.

VTG- and VLDL-associated xenobiotic transfer

Vitellogenin and VLDL are the primary lipoproteins sequestered by maturing oocytes of oviparous fish. While VLDL may plausibly be assumed to associate with greater molar amounts of lipophilic xenobiotics, VTG is typically produced and sequestered in far greater quantities than VLDL. Examination of LP-associated maternal transfer, therefore, must acknowledge that binding and transport are dependent upon a multiplicity of factors, including, but not limited to, the physicochemical properties of ligand and lipoprotein, as well as the serum chemistry and synthesis and sequestration kinetics of vitellogenesis.

Central to the concept of VTG as a transporter of xenobiotics to maturing oocytes is the hypothesis that the lipid and ionic regions of VTG can bind xenobiotic compounds in significant quantities and with adequate affinity to allow for oocytic accumulation. The coexistence of lipid and ionic regions on VTG makes it a potential carrier molecule for a wide array of endogenous (e.g., hormones, vitamins, minerals, etc.) and exogenous compounds possessing diverse physicochemical properties. While VTG transport of many of these compounds has not been proven, Specker and Sullivan (1994) and Sullivan et al. (1989) presented data supporting the role of VTG as a transporter of hormones in plasma samples from coho salmon

(*Oncorhynchus kisutch*). Further, Babin (1992) identified lipoproteins (potentially VTG) as the main plasma vector for transport of thyroid hormones, and Cyr and Eales (1989) reported binding of thyroxine to a presumed lipoprotein. Tagawa and Specker (1993), however, have shown that cortisol is taken up by the oocytes in the absence of VTG and in molar ratios similar to serum levels, implicating simple diffusion as the mode of uptake.

Although many ubiquitous aquatic pollutants possess structural and chemical similarities to steroid and thyroid hormones (Specker and Sullivan 1994), data implicating the role of VTG as a carrier protein for exogenous compounds is limited. Babin (1992) and Cyr and Eales (1992) have conducted studies showing that plasma lipoproteins (including VTG) are the principle binding sites for thyroid hormones in fish plasma. In recent studies, the thyroid hormones thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) have been shown to associate with VTG and to accumulate in oocytes of gravid *Fundulus heteroclitus* (Monteverdi 1999). In addition, the highly charged phosphate groups of the phosvitin region of VTG are presumed to be responsible for the significant binding capacity of the molecule for ions such as calcium, magnesium, iron, copper, and zinc (Specker and Sullivan 1994; Ghosh and Thomas 1995). However, little is known about the role this binding may play in the transport of nutrient ions or pollutant metals to maturing oocytes.

Fish vitellogenins and other serum lipoproteins have been associated with a number of exogenous ligands including persistent organochlorine compounds (e.g., PCBs; Mohammed et al. 1990) and a number of pesticides, including DDT (Ungerer and Thomas 1996; Mohammed et al. 1990). Plack et al. 1979), toxaphene (Mohammed et al. 1990), and dieldrin (Skalsky and Gutherie 1977). A number of studies, including those by Monteverdi (1999), Ankley et al. (1989), and Westin et al. (1983), have illustrated an apparent correlation between the onset of vitellogenesis and the delivery of common aquatic pollutants to the ovaries of gravid (i.e., egg-producing) fish. Further, a study by Ungerer and Thomas (1996) suggested that serum lipoproteins are intimately involved with the ovarian accumulation of *o,p'*-DDT in the Atlantic croaker (*Micropogonias undulatus*).

While conclusive, mechanistic descriptions of maternal transfer of xenobiotics by VTG and other lipoproteins have yet to be provided, a growing body of data suggests that such transfer is likely and serves a dual purpose as an important mechanism of xenobiotic depuration from maternal stores. For example, Guiney et al. (1979), Vodicnik and Peterson (1985), and Ankley et al. (1989) have all shown that spawning can enhance the elimination of maternal stores of PCBs.

Factors left unaddressed here that may prove important in the study of maternal xenobiotic transfer include the oocytic accumulation of xenobiotics by simple diffusion, in association with uptake of non-lipidated proteins, or (in the case of many marine teleosts) during oocyte hydration. Further, it has not been established whether lipophilic xenobiotics are preferentially sequestered by oocytes or whether

their transfer is determined by simple lipid-partitioning kinetics. Finally, an issue that overlies the entire discussion of lipoprotein-associated maternal transfer is the effect species- and environment-specific factors may play in the composition, transport, and sequestration of VTG and VLDL. For example, because the major source of lipid (i.e., fat stores versus diet) used in lipoprotein synthesis can vary as a result of genetic or environmental factors (e.g., availability of food), rates of maternal transfer can reflect variability that complicates our ability to predict xenobiotic sequestration in oocytes. Further work will be necessary to delineate the specific roles lipoproteins play in xenobiotic transport and sequestration by maturing oocytes.

Direct xenobiotic uptake and disposition in early life stages

For lipophilic compounds, current information suggests that maternal routes of xenobiotic exposure may be relatively more important for early developmental stages than is direct uptake. However, exposure data and the indirect, but more comprehensive, toxicity database suggest that eggs are capable of direct uptake of xenobiotics from the water. It is likely that environmental contaminant levels, contaminant and egg location, contaminant characteristics, structural and chemical characteristics of the eggs' surface, and the length of time the embryos develop in the impacted area are important determinants of direct exposure. Many of the comparisons between maternal and direct exposure have yet to be made quantitatively in natural systems under relevantly complex exposure conditions.

Xenobiotic uptake into eggs by direct exposure is highly variable depending on the characteristics of the chemical. Trans-chorionic permeability values for the eggs of medaka (*Oryzias latipes*), for example, have been shown to range from 10.5 to 82.9% for diethylnitrosamine, dipropylnitrosamine, dibutyltin dichloride, tributyltin chloride, lindane, pentachlorophenol, and aldrin (Helmstetter and Alden 1995). Log K_{ow}s of the compounds, when regressed against permeability factors, exhibited an R^2 value of 0.96. The authors suggested that the amount of xenobiotic entering the egg from external exposure was controlled in large measure by the lipid solubility of the chemical even in the presence of a membrane permeable carrier. Lake trout eggs exposed to TCDD at 0, 10, 20, 40, 62, or 100 ppt (ng[^{3}H] TCDD/L water) in an acetone carrier for 48 hours attained TCDD concentrations in a dose-related fashion with levels approximately 3 times greater than the nominal exposure concentrations (Walker, Spitsbergen et al. 1991). These concentrations were maintained through the egg and sac fry stages. In a stepwise fashion for each concentration, coho salmon embryos exposed under daily static renewal conditions to 0, 6, 13, 29, 62, and 139 mg/L of methyl mercury hydroxide rapidly attained near steady state levels of mercury by 15 days (Devlin and Mottet 1992). The mercury concentrations attained were approximately 16 to 24 times greater than the mercury levels in the exposure water. Investigations with inorganic mercury (mercuric chloride) have demonstrated even greater uptake rates for the ricefish embryo (Heisinger and Green 1975). Studies examining selenium accumulation in fathead minnow eggs reported

that 24-hour-old eggs from adults reared for 1 year in artificial streams containing 10 mg selenium/L (waterborne and dietary) accumulated 16 mg/g selenium whereas control eggs amassed 1.2 mg/g selenium (Schultz and Hermanutz 1990). When control eggs were exposed to selenium via the water under like experimental dosage conditions for 24 hours, the eggs took up 0.56 mg/g selenium. The authors suggested from this data that selenium in eggs comes primarily from parental exposure rather than from waterborne exposure of the new eggs.

A few studies have examined xenobiotic uptake, accumulation, and elimination throughout early development. These investigations highlight a number of interesting differences between each of the life stages. Studies with TBT and the minnow *Phoxinus phoxinus* show that TBT uptake rate was considerably lower in embryos than in yolk-sac larvae (Fent 1991). A 91-hour exposure of embryos to TBT resulted in residues of 0.85 mg/g, while an additional 64 hours of exposure to larvae resulted in residues of 4.65mg/g. The author suggested that the difference in TBT accumulation rates between embryos and yolk-sac larvae were related to the fact that larvae absorb TBT via skin and gills while the chorion of the egg modulates absorption. Likewise, embryos lack blood circulation, limiting distribution of the TBT. The authors also suggested that the uptake of TBT does not follow a simple hydrophobicity model, probably as a result of such morphological and physiological determinants.

Bioconcentration experiments with lindane in early life stages of rainbow trout indicate significantly higher BCFs at the hatching and yolk sac stages as compared to early juveniles (Vigano et al. 1992). Similar findings were demonstrated for *p*-dichlorobenzene (Galassi et al. 1982), 1,2,3 trichlorobenzene and 1,2,4 trichlorobenzene (Galassi and Calamari 1983). Interestingly, both the uptake and depuration coefficients of lindane in rainbow trout were consistently higher for young juveniles than in the other early stages. These findings suggest that the later developmental stages have a greater inward and outward flux of chemicals than do eggs or yolk sac larvae. Such results are not confined to lindane, as the same phenomenon has been noted with dieldrin (Van Leeuwen et al. 1985) and the elimination phase of trichlorobenzenes (Galassi and Calamari 1983). The authors of the lindane study suggested that the higher BCF for hatching and yolk sac stages relative to juveniles may be related to decreased lipid content or increased gill function for older life stages. They demonstrated that total lipid content in early life stages dropped from 8.0% in the eyed egg to a low of 1.95% in the early juvenile. An attempt to correlate this to an age dependent decline in BCFs was only partially successful. It was suggested that perhaps changes in the actual lipid composition during larval development may be responsible. They supported this premise with findings by Galassi et al. (1982), which determined that neutral lipids were 49.9% of the total lipids in eyed eggs, whereas at hatching and in the early fry stage neutral lipids comprised 61.8 and 73.9% of the total. Other features, such as biotransformation were not examined.

Solbakken et al. (1984) examined the uptake, accumulation and elimination of ^{14}C phenanthrene, naphthalene, benzo(a)pyrene (BaP), and 2,4,5,2',4',5' hexachlorobiphenyl by eggs and newly hatched larvae of cod (*Gadus morhua*). For the 24-hour exposure, the maximal accumulation in eggs occurred for phenanthrene, while the lowest uptake was noted for PCB. Examination of the yolk and chorion from cod eggs exposed to ^{14}C-labeled phenanthrene revealed that most of the compound was associated with the yolk. For each of the 4 compounds, most of the radioactivity that accumulated in the eggs was transferred to the larvae upon hatching. Larvae similarly exposed for 24 hours also exhibited the greatest uptake with phenanthrene, as seen with the eggs. However, the order of accumulation was different for the other compounds; the PCB showed the second highest accumulation, followed by BaP, and lastly by naphthalene. In general, larvae accumulated much more of each compound (except naphthalene) than did the eggs for a corresponding treatment. The authors suggested that the low accumulation of PCB in eggs may be due to the low lipid content of cod eggs and the high molecular weight of the PCB. The lower accumulation ranking of BaP in the larvae than in the eggs was attributed to increased metabolism in the larval stages, whereas the low accumulation and rapid elimination of naphthalene in both life stages was explained by a low lipid/water partition coefficient and high water solubility. Other studies have examined some of these same compounds in other species. Kuhnhold and Busch (1978) found that the relative accumulation of naphthalene and BaP in salmon eggs was dependent upon the duration of exposure. For short exposures naphthalene accumulated to a greater extent than did BaP; however, after 7 days the accumulation factor was 60% higher for BaP than for naphthalene. Both naphthalene and BaP were found to be associated with the yolk and vitelline fluid of the salmon egg (Kuhnhold and Busch 1978). Similar findings regarding distribution to the yolk have been reported for BaP in the sand sole egg (Hose et al. 1982). Investigations by Sharp et al. (1979) also have reported that the uptake of chrysene in killifish eggs was only 10% of that of naphthalene following a 2-hour exposure.

A variety of studies have examined the elimination of xenobiotics from early life stages of fish. For many compounds and fish species, xenobiotic elimination is a biphasic process through early development. Guiney and Peterson (1980) examined the elimination of the PCB 2,2',5,5' tetrachlorobiphenyl (TCB) during early development of rainbow trout. The half-life of 2,2',5,5' TCB was approximately 231 days in eggs and sac fry, while the compound was rapidly eliminated from fry (t_2 = 15 days). Other studies with PCBs have demonstrated this general theme. PCB losses from striped bass eggs and during stages of yolk absorption were variable; however, the loss of PCB appeared to increase rapidly once the yolk-sac was absorbed and the larvae began feeding (Westin et al. 1983). Even with continued dietary PCBs, larvae demonstrated a consistent reduction of parentally imparted PCB burdens (Westin et al. 1983). Striped bass larvae lost 78 to 97% of the initial egg PCB concentrations after 20 days of feeding. The PCB losses were most notable on a mg/g basis and were far less dramatic on a per larvae basis, suggesting that at least part of the apparent

loss was due to growth dilution. A similar reduction in PCB body burden and in concentration was observed for post-yolk-sac lake trout (Mac and Seelye 1981).

Elimination studies with other compounds in early life stages of other species parallel the studies with PCB. The elimination of TCDD from lake trout eggs and sac fry was very slow, with the absolute amounts of TCDD remaining relatively constant. Once reaching the fry stage (no yolk sac), TCDD was rapidly eliminated (t_2 = 35 to 37 days) in terms of absolute amounts (Walker, Spitsbergen et al. 1991). Studies with TBT and the minnow *Phoxinus phoxinus* show that elimination of TBT was very slow in all stages up through the yolk-sac stage, at which point elimination increased (Fent 1991). Rainbow trout eggs statically exposed to either 10 mg/L of 2,2'-dichlorobiphenyl (DCB) or 200 mg/L of 2,4,6-trichlorophenol (TCP) demonstrated biphasic elimination curves which in large measure correlated to life stage and the feeding process (Freitag et al. 1991). During the yolk-sac stage, elimination of both compounds was slow, while in the feeding larval stage, a rapid increase in depuration rate was noted. The elimination rate was higher on a wet-weight basis than on a per-animal basis. This again suggests that at least part of the apparent elimination was due to growth dilution. Dilution due to growth has been suggested in other studies for larval lake trout (Berlin et al. 1981) and fathead minnows (Defoe et al. 1978).

While biphasic elimination of xenobiotics is a prominent feature in early life stages of fishes, other processes are also operative. Trout embryos exposed at 1, 15, and 25 days post-fertilization to a 24-hour waterborne dose of [^{14}C] benzo(a)pyrene (BaP) demonstrated differential kinetics dependent on stage of exposure (Kocan and Landolt 1984). As long as the egg to volume ratio remained constant, the amount of BaP taken up by the eggs was consistent among all exposure-time treatments. In contrast, the elimination of BaP from the developing egg was highly dependent upon the stage at which the embryo was exposed. Embryos exposed prior to blastogenesis (27 to 30 days for depuration) lost only 2 to 3% of the initial concentration in the egg, while 15 to 20% was lost during a 5-to7-day period for the late exposed animals. Those embryos exposed prior to blastula formation, upon hatching, retained 80% of the initial BaP dose in the sac fry. Those dosed later retained approximately 60%. Such studies suggest that perhaps the time of exposure may influence the accessibility of the contaminant for elimination. Likewise, as has been shown for chemical uptake, other work suggests that compound character may be strongly influential in the early-life-stage elimination process. Solbakken et al. (1984) examined the elimination of ^{14}C phenanthrene, naphthalene, benzo(a)pyrene, and 2,4,5,2',4',5' hexachlorobiphenyl by eggs and newly hatched larvae of cod (*Gadus morhua*). Naphthalene was rapidly eliminated from eggs, while phenanthrene was slowly eliminated BaP and PCB showed no apparent elimination from the eggs after 12 hours in freshwater.

The foregoing studies provide some recurring themes regarding chemical uptake and disposition in early developmental stages. It is clear from the existing data that

early life stages may obtain a contaminant burden from both maternal sources and by direct exposure of the life stage to the contaminant in the environment. It is also evident that a variety of compounds can bioaccumulate in embryos and larval stages above ambient exposure levels. The compounds that show the greatest propensity to bioaccumulate, as in adults, appear to be those that are more lipophilic. Studies that have examined chemical distribution in early life stages indicate that contaminants were largely associated with the yolk. Several studies suggest that a greater chemical flux occurs with later development. It is unknown if this is due to development of uptake and depuration pathways, loss of contaminant storage areas in the form of yolk loss, developing competency of metabolic systems, or by some other factors. It is recognized that elimination rates from embryos and yolk-sac larvae are generally slow as compared to rapid increases in the feeding post-yolk-sac stage. There are numerous indications that a substantial portion of these apparent losses are due to growth dilution rather than actual elimination.

Biotransformation: P450 induction and in vitro activities with prototypic substrates

Much of the work with biotransformation in early life stages has focused upon P450 activity and induction as measured by prototypic substrates. Early ontogenic studies with killifish and brook trout demonstrated P450 mediated biotransformational activities (aryl hydrocarbon hydroxylase [AHH]) and their induction in both embryos (eggs pre-hatch) and in larvae (post-hatch) following exposure of embryos to oil or to PCBs (Binder and Stegeman 1980, 1983). Further investigations with hatchery and PCB-contaminated lake trout embryos and swim-up fry correlated contaminant burdens with P450 activity (Binder and Lech 1984). In these investigations, AHH activity was approximately 5-fold higher in lake trout embryos from Lake Michigan and Green Bay parental stock when compared to hatchery controls. Likewise, AHH activity was 4-fold higher in swim-up fry from contaminated sites than from hatchery derived stock. The total PCB burdens for swim-up stages were 0.175, 4.30, and 2.19 mg/g for hatchery, Lake Michigan and Green Bay swim-up fry, respectively. The authors suggested that burdens of contaminants imparted to progeny during maternal development resulted in induction of monooxygenase activities in these early life stages. When juvenile lake trout were allowed to depurate contaminants out to 210 days post-hatch in a control-laboratory situation, the PCB levels in contaminated Lake Michigan animals dropped to near-control levels, as did AHH activities. P450 activities as measured by hepatic AHH activity in control swim-up fry of lake trout were 3-fold higher than those of control embryos. Similarly, Binder et al. (1985) demonstrated that in killifish, embryos have a very low basal cytochrome P450-dependent enzyme activity. An interesting feature of these latter studies is that induction of activity, while present in all life stages, occurred at lower inducer concentrations for the eleutheroembryos (yolk-sac larvae) than for the embryos (Binder et al. 1985). The maximal activities attained, however, were much the same when exposed at higher PCB dosages.

Monod et al. (1996) expanded upon earlier studies by adding a diversity of biotransformation reactions and fish species. P450-mediated 7-ethoxyresorufin-O-deethylase (EROD) activity along with NADPH-cytochrome-c-reductase and glutathione-S-transferase (GST) activity was examined in arctic charr (*Salvelinus alpinus*), whitefish (*Coregonus lavaretus*), and grayling (*Thymallus thymallus*). In these studies, enzyme activities increased during embryolarval development for all 3 species. The rate of increase of EROD activities in whitefish, grayling and arctic charr was 3-fold higher following hatching, while NADPH-cytochrome-c-reductase activity appeared to reach maximal rates of increase prior to hatching. GST activity appeared to mirror that of EROD in the grayling and whitefish, while the charr exhibited an increase in catalytic rate prior to hatching. EROD, NADPH-cytochrome-c-reductase, and GST activities in subcellular fractions prepared from arctic charr embryos were 0.00124, 2.03, and 50 nmol/min/mg protein, respectively. In eleuteroembryos the EROD, NADPH-cytochrome-c-reductase, and GST activities were higher, with values of 0.0047, 2.53, and 90 nmol/min/mg protein, respectively. Exposure of arctic charr embryos and eleuteroembryos for 72 hours to 0.12 ppm of the inducer β-naphthoflavone (BNF) in the water resulted in a 6-fold induction of EROD for both life stages and no change for either NADPH-cytochrome-c-reductase or GST.

EROD activity has been measured in larval, juvenile, and adult turbot (*Scophthalmus maximus* L.) following exposure to contaminants and model inducers (Peters and Livingstone 1995). In the turbot, basal EROD activity was not measurable in embryos, but it increased for 3-day larvae through adults. The activities were 0.45, 0.57, 10.8, and 12.3 pmol/min/mg protein for 3-day larvae, 11-day larvae, 90-day juveniles, and adults, respectively. Exposure of 4-day turbot larvae for 24 hours to 5 ppb BaP increased whole-body EROD activity 3-fold, while hepatic EROD activity following exposure to 25 ppb BaP for 48 hours was elevated 2-fold in 90-day juveniles and 13-fold (BNF injection) in adults. Seven-day turbot larvae exposed to lindane for 48 hours demonstrated a 6-fold increase in whole-body EROD activity.

Induction studies have been performed with cod larvae and juveniles following differing exposures to the water soluble fraction (WSF) of North Sea Crude Oil (Goksoyr et al. 1991). Measurement of P4501A1 by an ELISA assay indicated that exposure to levels of WSF as low as 40 mg/L elevated P4501A1 levels in larvae. Even though the exposure was started during the early egg stage, in all of the larval experiments induction was delayed until after hatching. WSF which contains predominately compounds such as benzene, toluene and xylene resulted in P4501A1 induction in juvenile cod as evidenced by EROD, western blot and ELISA assays. When exposed juveniles were transferred to clean water P4501A1 levels dropped to 85% and EROD activities dropped to 64% of exposed animals within 2 days.

Investigations examining waterborne 3,3',4,4' tetrachlorobiphenyl (TCB) accumulation in gonads, liver, and muscle of adult fathead minnows, as well as immunohistochemical localization of P4501A in the adult and F1 larval fishes, suggests that

TCB transfer to larval fishes may result in P450 induction in those early life stages (Lindstrom-Seppa et al. 1994). The major sites of induction in post-hatched larvae were in the endothelium of yolk-sac vessels and branchial vessels. This may be a result of localized dosimetry as PCB was mobilized from the lipid storage depots in the yolk sac. In studies with rainbow trout eggs, where an inducer (BNF) was injected directly into the egg, CYP1A was strongly induced, particularly in cellular sites that would have been induced in the adult (Lauren et al. 1990). Lake trout eggs injected with TCDD also demonstrate strong induction of P450-dependent activities in multiple cell types in embryos (Guiney et al. 1992).

Taken together, the results of the foregoing studies suggest a number of important features regarding P450 induction and Phase I biotransformation in early life stages. The first being that Phase I xenobiotic metabolism may occur in both the embryo and the larval stages. Basal levels of P450 activity are generally higher in larval stages when compared to the low levels present in embryos. Eggs and larvae may be induced environmentally, through maternal routes, and by experimental routes such as injection. Although CYP1A is inducible before and after hatching in fish, there appears to be an increased sensitivity in the induction response after hatching. P450 activity in eggs and larvae, while inducible, is transitory at induced levels when the source of the inducer is removed. Levels of P450 activity are considerably lower in embryo, yolk-sac, and early swim-up stages than in adults following similar treatments. Potential etiologies for differences in biotransformation and induction response between life stages could be multifold. The fact that eggs respond at higher concentrations and the strong response in eggs upon injection suggest that dosimetry considerations relative to the inducer may play an important role in these findings. Obvious issues such as permeability of the egg chorion, the high lipid reserves in eggs, lack of a defined circulation in the egg, and changes in diffusive surface area upon hatch may be operative factors. Furthermore, it has been suggested that variation in the responsiveness of the Ah receptor may occur among the various life stages (Stegeman and Hahn 1994). Very little is known about both the dosimetry and molecular aspects of P450 induction in early life stages. As is evident from this discussion, even less is known regarding ontogeny, induction, and activity of Phase II reactions in developmental stages.

In vivo biotransformation by early life stages

A limited number of studies have examined in vivo xenobiotic metabolism in early life stages of fish. Eggs or embryos appear to exhibit, by the methods employed, limited in vivo biotransformation. Studies examining metabolism of TBT in the embryos of the minnow *Phoxinus phoxinus* indicated that metabolism, as indicated by the appearance of metabolites in the water, was very low or absent (Fent 1991). Likewise, Sharp et al. (1979) reported that killifish embryos did not metabolize either naphthalene or chrysene when the water was examined. Extracts of lake trout eggs following exposure to TCDD also contained only parent compound with no metabolites being detected (Walker, Spitsbergen et al. 1991). In contrast to the

foregoing studies, trout embryos exposed to a 24-hour, waterborne dose of ^{14}C benzo(a)pyrene excreted water soluble products into water (Kocan and Landolt 1984). Approximately 30 to 45% of the metabolites were extractable with ethyl acetate (phenols and diols) and 60% were conjugated products. However, there was no quantitative indication of how much of the initial BaP dose was metabolized.

Evidence for in vivo xenobiotic metabolism is also inconsistent for the latter stages of early fish development. The in vivo metabolism of aniline and pentachlorophenol (PCP) by Arctic charr eleutheroembryos at the end of yolk-sac resorption were examined by Cravedi et al. (1995). Following a 48-hour static exposure to PCP, 18.3, 24.2, and 49.4% of the radioactivity in water was found as PCP, PCP-glucuronide, and PCP-sulfate, respectively. This compositely amounted to 13.7% of the administered radioactivity. A total of 51.9% of the administered radioactivity was recovered from water following aniline exposure. Parent compound accounted for 13.8%, while acetanilide accounted for 76.4% and p-aminophenol 2.1%. The authors presented this data as evidence that early life stages of salmonids were capable of biotransformation reactions such as N-acetylation, sulfation, and glucuronidation.

Biotransformation of dietary hexachlorobenzene (HCB) was examined in steelhead trout fry (Frankovic et al. 1995). In 1 gram fry, there was evidence of reductive dechlorination, as traces of 2,5-dichlorophenol, 2,3,6-trichlorophenol, and 2,4,5 trichlorophenol were evident in fry extracts late in the depuration phase. However, no metabolites including pentachlorophenol or its conjugates were evident earlier in the time course. HCB in 10g fry was metabolized to low levels of pentachlorophenol and its glucuronic acid conjugate. Biotransformation of tributyltin has been demonstrated to be very slow or absent in yolk-sac larvae (Fent 1991). Additional studies suggest that TBT may in fact inhibit its own metabolism (Fent and Stegeman 1991). As in the embryo, metabolites of TCDD were not detected in sac-fry stages of lake trout (Walker, Spitsbergen et al. 1991).

The in vivo studies with early life stages appear less consistent in demonstrating biotransformation than in vitro experiments using prototypic substrates. What is an uncertainty in the existing knowledge base is what role analytic sensitivity and differential metabolite solubility play on the detectability of inherently low levels of metabolites. The small body size, whole body, and water dilution of metabolites, as well as apparently low-basal, uninduced metabolic rates, complicate assessment of biotransformation in vivo. Biotransformation by eggs or larvae with their low metabolic rates (P450) may well go undetected because of these factors. In eggs, transchorionic movement of both parent and metabolites and xenobiotic partitioning into lipid stores are added processes that may play a modulating role in vivo, but not in vitro. In general, more work will have to be performed on kinetic and biotransformational issues before the determinant factors and relative importance of real-world early-life-stage biotransformation can be ascertained.

Chemical disposition in birds

Overview

Accumulating evidence suggests that a variety of compounds have adversely affected reproduction and development in wild birds. Included among these compounds are DDT and related metabolic products (Ratcliffe 1967; Hickey and Anderson 1968; Cooke 1973), PAHs (Kubiak et al. 1989; Gilbertson et al. 1991; Harris et al. 1993; Hoffman et al. 1993; Larson et al. 1996), methylmercury (Barr 1986), selenium (Ohlendorf et al. 1986), and crude oil mixtures (Ainley et al. 1981; Trivelpiece et al. 1981; Fry et al. 1986). Case studies detailing some of these effects and the mechanisms thought to underlie them are described in Chapter 4 of this volume. For additional information, the reader is referred to several excellent reviews (Giesy et al. 1994; Fry 1995; Barron et al. 1995), as well as a text on interpretation of contaminant residues in wildlife (Beyer et al. 1996).

The response of the scientific community to these observations has been impressive. A survey of literature published in the last 5 years shows that residue levels have been characterized in birds and eggs from numerous contaminated sites, revealing both temporal and geographic trends (e.g., Ormerod and Tyler 1994; Hebert et al. 1994; Hothem et al. 1995). Embryotoxic effects have been described for eggs collected in the field (Harris et al. 1993; Hoffman et al. 1993; Sanderson et al. 1994; Larson et al. 1996) and after maternal dosing in the laboratory (Heinz and Hoffman 1996; MacLellan et al. 1996; Summer et al. 1996; Sanderson et al. 1997). A variety of compounds have been injected directly into eggs to study metabolic biotransformation as well as toxicity (e.g., Nosek et al. 1993; Van Den Berg et al. 1994; Sanderson and Bellward 1995; Janz and Bellward 1996a, 1996b; Sanderson et al. 1997; Zhao et al. 1997), and kinetic studies have been performed to characterize the transfer of accumulated residues from adults to eggs and from eggs to chicks (Nichols et al. 1995; Custer and Custer 1995).

Despite this progress, however, relationships among environmental exposure, absorbed dose, tissue concentration time-course, and toxic effect are seldom known. Traditionally, contaminant levels in wildlife have been related to residues which, on the basis of laboratory or other data, are thought to be toxic (Beyer et al. 1996). These comparisons are often complicated by differences in dosing route, dose level, and exposure duration. Environmental exposures are in general more complex than those carried out in the laboratory, due to fluctuating concentrations and natural history considerations (e.g., migration and changes in dietary preference). Residue levels in field-collected specimens must therefore be viewed as the integrated result of numerous factors, many of which may be poorly understood. Quantitative models embodying a variety of approaches have been used extensively in environmental toxicology to describe contaminant uptake and disposition (reviewed by Landrum et al. 1992; Newman 1995). Unfortunately, efforts to develop such models for birds have lagged considerably behind similar efforts with fish and mammals. It

is reasonable to expect that basic toxicokinetic principals established in studies with other taxa would apply also to birds. However, because so little information exists, any discussion of chemical kinetics in birds must be labeled as informed speculation.

The goal of this section is to review factors that could have a large impact on chemical uptake and disposition in birds. Consistent with the goals of this volume, special emphasis is placed on attributes of reproducing adults and their developing offspring. The following topics are discussed in the sections below.

- A general discussion of routes of exposure and other factors relevant to adult birds;
- Metabolic biotransformation, with an emphasis on the metabolic capabilities of developing embryos;
- The reproductive biology of birds, in relationship to maternal transfer of contaminants and the disposition of compounds in eggs and juveniles;
- Two kinetic models for birds, and;
- Suggestions for future research, including the proposed development of linked bioenergetics and physiologically based kinetic models.

Biological attributes of adults that may impact exposure assessment

The diet is the most important route by which adult birds are exposed to toxic compounds. Direct chemical application as a result of, for example, airborne spraying of a pesticide, could lead to a significant dermal exposure, although even in this instance the oral route may predominate because of preening behaviors. The consumption of contaminated water could also contribute to the total applied dose, particularly in exposures to metals and metalloids. Inhalation is unlikely to represent an important route of uptake except in exposures to highly contaminated atmospheres, occurring when animals live within the exhaust plume of an industrial discharge.

Historically, demonstrated impacts on raptorial and piscivorous birds have focused attention on the importance of chemical bioaccumulation and biomagnification. Chemical attributes that contribute to bioaccumulation include environmental persistence and hydrophobicity . Bioaccumulation in aquatic biota is commonly expressed using a bioaccumulation factor (BAF), which is defined as the concentration of a substance in the animal, accumulated by all possible routes, divided by that in water. BAFs exceeding 10,000 are common for hydrophobic compounds that do not readily undergo metabolic biotransformation. Bioaccumulation can also be referenced to a chemical concentration in aquatic sediment. In general, biota-sediment accumulation factors (BSAFs) are much lower than BAFs, owing to the substantial chemical capacity of most sediments. Chemical bioaccumulation also occurs in terrestrial systems. In such cases, residues are generally referenced to chemical concentrations in soil or in dietary constituents. Biomagnification is defined as a progressive increase in chemical concentration at successively higher

trophic levels, and it is always associated with a predominantly oral route of exposure. Biomagnification occurs in both aquatic and terrestrial food webs. By definition, biomagnification is associated with an increase in the BAF at each trophic level. Together, bioaccumulation and biomagnification can result in substantial delivered doses to animals that feed at the top of either aquatic or terrestrial webs.

Attention has also been given to birds that feed at intermediate trophic levels. American robins (*Turdus migratorius*) were shown to accumulate substantial residues of DDT and other pesticides after consuming contaminated earthworms (Johnson et al. 1976; Beyer and Gish 1980). More recently, attention has been focused on species that consume emergent aquatic insects, including both tree swallows (*Tachycineta bicolor*) and red-winged blackbirds (*Agelaius phoeniceus*) (DeWeese et al. 1985; Ankley et al. 1993; Nichols et al. 1995; Bishop et al. 1995). Birds also can consume toxic levels of some compounds without a requirement for food web effects or bioaccumulation. One notable example is the ingestion of granulated pesticides by granivorous species (USEPA 1992). In general, however, granivorous birds are less likely to accumulate high body burdens of persistent environmental contaminants than insectivores, piscivores, or carnivores (Enderson et al. 1982; Elliot et al. 1994).

Field surveys suggest that the extent of chemical biomagnification in piscivorous birds usually exceeds that of predatory fish. For example, biomagnification factors (BMFs) ranging from 2 to 10 were reported for a variety of persistent organic compounds in large salmonids from Lake Ontario (Connolly and Pedersen 1988; Oliver and Niimi 1988). Herring gulls (*Larus argentatus*) feeding on the same prey base exhibited BMFs ranging from about 10 to 200 (Clark T et al. 1988; Braune and Norstrom 1989). The reason for this difference is not entirely clear, although it can be speculated that the absence in birds of branchial elimination is a contributing factor. Methylmercury may represent an exception to this general rule. BMFs of approximately 7 were observed in common loons (*Gavia immer*) and herring gulls feeding on rainbow smelt (*Osmerus mordax*) and blunt nosed minnows (*Pimephales notatus*) (Wren et al. 1983). Common mergansers (*Mergus merganser*) feeding on yellow perch exhibited a BMF of 2.5 (Vermeer et al. 1973). BMFs of 2 to 10 are commonly reported for large predatory fish (e.g., Wren et al. 1983; Cope et al. 1990; Lindqvist 1991; Mason and Sullivan 1997). Unlike persistent organic compounds, methylmercury can be eliminated from birds by incorporation into feathers (Braune and Gaskin 1987). Cadmium and selenium have also been shown to deposit in feathers, leading to the use of feathers as non-invasive bioindicators of exposure (Bowerman et al. 1994; reviewed by Scheuhammer 1991; Burger 1993).

Limited data suggest that the oral bioavailability of lipophilic compounds in birds is highly variable and depends upon both the dosing vehicle and the species. For example, the bioavailability of 2,3,7,8-TCDD ranged from 30% (earthworm suspension) to 58% (cricket suspension) when fed to hen pheasants (*Phasianus colchicus*)

(Nosek et al. 1992). Oral bioavailabilities of 90% were observed when Arochlor 1254 was loaded into a gelatin capsule and fed to pigeons (*Columbia livia*) (de Freitas and Norstrom 1974) or spiked into grain and fed to pheasants (Dahlgren et al. 1972). Using an in vitro perfusion technique, Serafin (1984) found that intestinal absorption of 2,2',4,4',5,5'-hexachlorobiphenyl, dieldrin and mercury (as $HgCl_2$) varied greatly among 5 bird species and suggested that these differences could result in dissimilar uptake in intact animals. Unfortunately, the oral bioavailability of most chemical classes in adult birds is essentially unknown, and no data are available for young birds of any species.

Energy metabolism in birds is reviewed by Whittow (1986). Birds are largely homeothermic, although many smaller species allow core temperatures to drop for short periods of time as a means of conserving energy. Standard metabolic rates in non-passerine birds are similar to or somewhat higher than those of placental mammals of similar size, while those of passerine birds may be a factor of 2 higher (McNab 1988; Daan et al. 1990). Metabolic rates across species tend to scale to a fractional exponent of body weight (about 0.7 to 0.8 in adults), resulting in marked differences in weight-adjusted metabolism and consumption. A potential consequence of this fact is that small birds ingesting large quantities of moderately contaminated prey can, within a similar period of time, consume the same dose (on a weight-adjusted basis) as a larger bird eating relatively smaller quantities of more highly contaminated prey.

Migration, courtship, breeding, and parental caregiving behaviors require high expenditures of energy and are often accompanied by periods of starvation. Birds respond to these situations by mobilizing stored lipid. In such cases, toxicity may occur as a result of the release of lipophilic contaminants that, prior to that time, had been sequestered in body-fat stores. Mirex concentrations in brain tissues of 4 species of passerine birds increased during a period of food depletion, resulting in the attainment of lethal residues (Stickle et al. 1973). Similar findings were reported for cowbirds (*Molothrus ater*) and American kestrels (*Falco sparverius*) exposed to DDT and DDE, respectively (VanVelsen et al. 1972; Porter and Wiemeyer 1972). DDE concentrations in brain tissues from sparrowhawks (*Accipiter nisus*) collected in the field were found to be inversely related to whole-body lipid status (Bogan and Newton 1977). Whole-body concentrations of DDE and total PCBs increased in herring gulls during seasonal periods of lipid depletion (Anderson and Hickey 1976). A net movement of PCBs from adipose fat to muscle occurred in pigeons during starvation (de Freitas and Norstrom 1974); upon subsequent feeding, PCBs moved back into fat.

Metabolic biotransformation

Adults

A bird's ability to eliminate a compound once it has been absorbed depends on the attributes of the compound and of the bird itself. In general, relatively polar, organic

compounds can be efficiently eliminated in urine or feces. Relatively non-polar compounds tend instead to partition to somatic lipid stores and are eliminated very slowly unless they can be transformed to more polar products. Enzyme systems capable of metabolically transforming xenobiotic compounds exist in many tissues, although enzyme activities tend to be highest in organs of elimination such as the liver and kidney. Because it facilitates elimination, metabolic biotransformation often leads to detoxification of the substance in question. It is important, however, to recognize that in some instances these biotransformation reactions create products that are more toxic than the parent compounds. This outcome, referred to as bioactivation, has attracted a great deal of attention because of the potential for unanticipated toxicity, including effects (e.g., carcinogenesis) that occur long after the initial exposure.

A comprehensive review of metabolic biotransformation in birds is beyond the scope of this chapter. Nevertheless, several generalizations may be attempted regarding metabolism in adult birds. Constitutive levels of metabolic enzymes and the activity of these enzymes toward a variety of substrates have been determined in tissues from several species (reviewed by Pan and Fouts 1978; Ronis and Walker 1989; Walker and Ronis 1989; Walker, Brealey et al. 1991). In general, mixed function oxidase (MFO) activities are highest in the liver. Substantial activity also may be present in the kidney, possibly due to the existence of a renal portal shunt that carries blood from the gastrointestinal tract to the kidney (Pan and Fouts 1978). The cytochrome P450 content of hepatic microsomes varies greatly among species but is, on average, about one-quarter that of most mammals. Differences among species may in some cases be related to feeding ecology. The content and activity of oxidative enzymes are in general higher in omnivorous birds than in piscivorous species. Indeed, enzyme activities in several species of seabirds appear to be similar to those of the fish that they consume (Walker and Knight 1981).

The activities of specific P450 isoforms are generally lower than those of mammals. Gender-related differences in enzyme activity have been noted (Knight and Walker 1982; Walker and Ronis 1989), although in other studies there were no differences between sexes (Knight and Walker 1982; Husain et al. 1984; Peakall et al. 1986). Biotransforming capabilities can in some cases be determined from chemical residue patterns in field-collected birds. For example, the metabolism of specific PCB congeners has been inferred by comparing congener patterns in tissues to those of defined Aroclor mixtures (Borlakoglu et al. 1990a, 1990b, 1990c, 1991). Alternatively, PCB congener levels can be expressed in relationship to the concentration of a congener which, on the basis of other information, is thought to be poorly metabolized (Borlakogu et al. 1990c). Both approaches have been used to establish structural "rules" for metabolism of PCBs by birds, based on the number and position of chlorine substituent groups (Borlakoglu et al. 1990c). Controlled laboratory studies have, for the most part, provided support for these generalizations (de Freitas and Norstrom 1974; Rozemeijer et al. 1995).

In birds, as in mammals, a large number of chemicals have been shown to induce metabolic enzyme activity, leading to the proposal that induction can be used as a biomarker for exposure (Payne et al. 1987; Rattner et al. 1989; Ronis and Walker 1989). Interestingly, variation in the inducibility of specific enzymes tends to be greater among adults than among embryos and juveniles of the same species (Rattner et al. 1989). Additional studies have explored the role of metabolism in determining species sensitivity, either by affecting the kinetics of a toxic parent compound or by creating toxic levels of a reactive metabolite (Walker 1978; Knight et al. 1981; Knight and Walker 1982; Walker et al. 1987; Walker, Brealey et al. 1991).

Embryonic metabolism

In contrast to mammals, birds exhibit high levels of MFO activity during the embryonic and neonatal periods. In chicken embryos (e.g., white leghorn, *Gallus domesticus*), AHH activity 5 days after fertilization (the earliest point at which an embryo can be sampled) is comparable to adult levels (Hamilton et al. 1983). Similar findings have been reported in herring gulls (Boersma et al. 1986; Peakall et al. 1986) and black-crowned night herons (*Nycticorax nycticorax*) (Hoffman et al. 1986). High levels of MFO activity are maintained and may even increase somewhat during the first few days after hatching, declining slowly thereafter (Powis et al. 1976; Haug et al. 1980; Brunstrom 1986).

AHH activity in the chicken embryo is inducible with 3,3',4,4'-tetrachlorobiphenyl (IUPAC no. 77) as early as 5 days after fertilization (Hamilton et al. 1983). A partial listing of compounds that have been shown to induce enzyme activity in ovo includes 3,3',4,4',5-pentachlorobiphenyl (IUPAC no. 126), 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC no. 169), 3-methylcholanthrene, phenobarbital, allylisopropylacetamide, β-naphthoflavone, and TCDD (Mitani et al. 1971; Poland and Glover 1977; Rifkind et al. 1982, 1985; Brunstrom 1986, 1991; Brunstrom and Andersson 1988; Sanderson and Bellward 1995; Janz and Bellward 1996b; Sanderson et al. 1997). The extent of enzyme induction has been correlated with contaminant residues from field-collected eggs (Hoffman et al. 1987; Bellward et al. 1990; Sanderson et al. 1994, Van Den Berg et al. 1994). Correlations have also been reported between AHH induction and hatching success (Hoffman et al. 1987). A comparison of data from 4 bird species suggested that the extent of EROD induction in ovo by TCDD was related to differences in Ah receptor affinity (Sanderson and Bellward 1995). The potential toxicological significance of embryonic metabolism was demonstrated by injecting a mixture of PAHs into eggs of the chicken on day 4 of incubation. By day 18, greater than 95% of all PAHs taken up by the embryo had been metabolized (Naf et al. 1992). Hydroxylation of 3,3',4,4'-tetrachlorobiphenyl by the chicken embryo yield products that, in comparison to the parent compound, are much less toxic and exhibit lower affinity for the Ah receptor (Wehler et al. 1990).

Aspects of reproductive biology that may affect exposure assessment

Maternal transfer

The process of vitellogenesis and protein deposition in bird eggs is similar to that described previously for fish (Verrinder Gibbins and Robinson 1982; Wallace 1985). Environmental cues give rise to endocrine-modulated synthesis of VTG and other lipoproteins in the liver. These substances are then transported by the circulatory system to the ovary and selectively sequestered as critical components of oocyte maturation. In ovo proteolytic cleavage of these lipoproteins provides for oocyte growth and is the primary nutrient source for the embryonic bird. Unlike the early-life stages of many fish species, however, yolk ceases to be a nutrient source soon after hatching.

From the standpoint of lipoproteins functioning as transporters of xenobiotic compounds, the most notable difference between birds and non-avian oviparous vertebrates lies in the source of lipids for the maturing oocyte. While VTG is the source of the vast majority of yolk lipid in fishes, amphibians, and reptiles, 80 to 90% of the protein-associated lipid in avian oocytes comes from VLDL (Hillyard et al. 1956; Wallace 1985). Furthermore, on a dry-weight basis, VLDL is the major yolk-related protein in birds (Schjeide 1954; McIndoe 1959).

By virtue of its greater lipidation, it can be speculated that VLDL has a greater capacity to transport lipophilic xenobiotics than VTG. However, Borlakoglu et al. (1989) reported that partitioning of various PCB congeners into serum lipoprotein fractions (VLDL, LDL, and HDL) cannot be predicted based solely on solubility in the lipid components of these fractions. The possibility also exists that compounds may bind to VTG and VLDL in a specific manner, facilitating their deposition in the egg. Studies on the maternal transfer of calcium (Packard and Clark 1996), estradiol (Adkins-Regan et al. 1995), and immunoglobulins (Lung et al. 1996) suggest the importance of this mechanism in determining both egg composition and successful embryogenesis. In general, however, the extent that lipoprotein participates in the oocytic sequestration of both endogenous and xenobiotic compounds in avian species has yet to be clearly defined.

Chemical residues in eggs and tissues from the same maternal parent have been shown to be statistically correlated (Mineau 1982). Similar correlations exist when eggs from the same nest are compared to eggs from different nests (Thompson et al. 1977; Custer et al. 1990). When normalized for lipid content, DDE levels in eggs and carcasses of black ducks (*Anas rubripes*) were essentially identical (Longcore and Stendell 1977). A similar result was reported for DDE in sparrowhawks (Bogan and Newton 1977). Collectively, these observations suggest that a chemical equilibrium is established between a female and its eggs before the eggs are laid.

In other cases, an internal equilibrium does not appear to have been achieved. Lipid-normalized PCB residues in eggs and liver from herring gulls were about half the levels measured in adipose tissue (Norstrom, Clark, Jeffrey et al. 1986). It was

suggested by these authors that diet, rather than adipose tissue, was the main source of lipid deposited in eggs. Residue levels in eggs thus came to resemble those of the liver, which is the organ within which yolk lipids are synthesized and packaged. Low chemical concentrations in the liver (lipid-normalized) were thought to be due to dilution of existing lipid stores with "new" lipid absorbed directly from the digestive tract or synthesized from dietary protein.

Work with several bird species supports the suggestion that lipids required for egg formation derive primarily from dietary sources (Roudybush et al. 1979). However, the extent to which this observation can be generalized to all birds is unclear. Moreover, the relative contribution of stored and dietary lipid may change with body condition and food availability, and perhaps even within a single clutch of eggs. Thus, Mineau (1982) found that organochlorine contaminant levels increased in sequentially laid herring gull eggs, consistent with an increase in lipid content.

There is also a lack of consensus on whether egg laying represents an important route of chemical elimination for female birds. Nosek et al. (1992) found that female pheasant transfer about 1% of their TCDD body burden to each of 15 sequentially laid eggs. Based on the observation that wild pheasant lay as many as 30 eggs a year, it was concluded that egg laying contributes substantially to chemical elimination. Sparrowhawks eliminated as much as 50% of their DDE burden in a single clutch of eggs (Bogan and Newton 1977), while Arctic terns (*Sterna paradisaea*) and herring gulls transferred 45% and 24%, respectively, of their maternal PCB load to eggs (Lemmetyinen et al. 1982).

It may not be possible, however, to determine the importance of egg laying as an elimination route from a simple comparison of residue masses (Σ all eggs/total in the parent). For example, Norstrom, Clark, Jeffrey et al. (1986) found that although 16% of [^{14}C] DDE was transferred from female gulls to eggs, the combined weight of the eggs comprised 28% of the total body weight. The concentration of DDE in the eggs was therefore considerably lower than that of the gulls. Under such circumstances, it may be speculated that egg laying results in an *increase* in whole-body residue concentration. Norstrom, Clark, Jeffrey et al. (1986) concluded that while important, egg laying accounted for only 15% of the total elimination of DDE. Taken together, these data suggest that the importance of egg laying as a route of chemical elimination is species-specific and depends on a number of factors including egg and clutch size, maternal fat reserves, and the extent to which these reserves are mobilized to provide for yolk deposition.

Data pertaining to maternal transfer of compounds other than lipophilic organics are extremely limited. Heavy metals, including lead and cadmium, have been detected in eggs of several bird species (Burger and Gochfeld 1991, 1993, 1995). However, relationships between metal concentrations in parents and their eggs are not commonly known. In a study of common terns, lead levels in eggs were positively correlated with levels in feathers from the maternal parent (Burger and

Gochfeld 1991), but were much lower overall. Cadmium levels in eggs were also much lower than those in feathers and did not exhibit a statistical correlation with maternal concentrations. The significance of these observations is difficult to determine. As indicated previously, several metals are eliminated from birds by incorporation into feathers. Comparing the metal concentration in an egg to that of a feather does not, therefore, provide a straightforward basis for comparisons with internal tissue levels. In the same study, lead levels were higher in feathers from female terns than in feathers from males. However, because of possible sex-related differences in dietary loading, this finding does not by itself indicate that eggs are unimportant as an elimination route for lead.

Interestingly, concentrations of some heavy metals may be higher in the shell portion of the egg than in the egg contents (Burger 1994). This is important, since many researchers analyze only the egg contents when they are reporting on maternal transfer of metals. The distribution of metal between the shell and the egg contents may also have toxicological implications for the developing embryo.

Selenium concentrations in eggs taken from waterfowl at a contaminated site were comparable to those in the livers of adult birds (Ohlendorf et al. 1986). Subsequent laboratory studies with mallard ducks (*Anas platyrhynchos*) confirmed that selenium levels in eggs and livers of breeding females are similar and highly correlated (Heinz et al. 1989). Correlations among selenium levels in other tissues have also been reported (reviewed by Heinz 1996). However, these levels can change rapidly as selenium levels in the diet change. In contrast to selenium, mercury levels in eggs from common loons were much lower than those in the livers or feathers of adult birds (Belant and Anderson 1990).

The interpretation of these findings is complicated by the fact that selenium and mercury do not partition with tissue lipid, but instead are incorporated into protein. The uptake and retention of both compounds are enhanced by prior transformation to organometallic forms (principally selenomethionine and methylmecury). As indicated previously, deposition into feathers represents a route by which birds eliminate mercury and selenium (Braune and Gaskin 1987; Bowerman et al. 1994). Hepatic demethylation may also play a role in the elimination of mercury. Evidence for demethylation is provided by the observation that mercury found in the liver often exists as Hg^{2+}, while in other tissues the predominant form is methylmercury. Studies suggest that the mechanism of demethylation depends upon the presence of selenium (Palmisano et al. 1995; Cavalli and Cardellicchio 1995), possibly explaining the protective effect of dietary selenium against methylmercury toxicity to birds (Ganther et al. 1972). Limited evidence also suggests that the extent to which this pathway is developed depends upon a bird's feeding habits. Among adult ducks, Fimreite (1974) found that fish-eating mergansers contained the lowest levels of methylmercury in liver (12% of total), while in goldeneyes, mallards, and pintails, methylmercury constituted 32, 38, and 52% of the total, respectively. Work by Fimreite (1974) also suggests that this detoxifying ability appears early in life.

Thus, methylmercury in livers taken from ducklings constituted 27, 49, 53, and 58% of the total in mergansers, mallards, goldeneyes and pintails, respectively. Methylmercury levels in breast muscle from all 4 species were essentially identical, averaging about 60% of total.

Embryonic development

Once an egg is laid, its protective shell limits chemical interactions between the embryo and its surroundings. Toxic substances applied directly to eggs may in some instances cause embryotoxicity and reduced hatchability (reviewed by Hoffman 1990; Hoffman and Albers 1984), but this occurs only rarely in the wild. In a majority of cases, chemical dose to the developing embryo is determined by maternal inheritance and subsequent internal events. Chemical residues in eggs are usually measured and expressed on a whole-egg basis. Limited data suggest that very early in development, hydrophobic organic compounds partition almost exclusively to yolk, due to its high lipid content (Nosek et al. 1992). Later, as embryos grow, these chemicals diffuse across the yolk membrane and redistribute to developing tissues and organs (Naf et al. 1992; Sanderson and Bellward 1995). It is unclear, however, whether this redistribution results in an equilibrium distribution among tissues. Because the egg is a closed system, this question may be of particular importance. As noted previously, contaminant concentrations in eggs may approach maternal levels when normalized for lipid content. During development, most of the yolk originally deposited in an egg is consumed to support the growth tissues which are, by comparison, relatively lean. Assuming an absence of metabolic biotransformation, chemical concentration within the egg must remain the same. The potential exists, therefore, for chemical concentrations in "lean" embryonic tissues to exceed those of the same tissues of the maternal parent.

Juvenile development

Avian physiologists frequently distinguish among species based on the extent of development of the young at hatching. Birds that are in an advanced state of development are called precocial, while those in an early stage of development are termed altricial. An examination of these two breeding strategies suggests several differences that could have toxicological and toxicokinetic consequences. Relative to the size of the adult bird, the eggs of altricial species are smaller than those of precocial species and contain less yolk. Altricial species do not thermoregulate upon hatching and can therefore utilize a higher percentage of ingested energy for growth than precocial birds. On the other hand, demands on the adults of altricial species may be greater due to relatively narrower limits for brooding behavior. Anecdotal evidence suggests that in several cases, toxicity to young birds was exacerbated by reduced quality of parental care (Fox et al. 1978; Kubiak et al. 1989).

Limited data are available on chemical kinetics in very young birds. Nosek et al. (1992) estimated the $t_{1/4}$ for whole-body elimination of [^{3}H]TCDD (total TCDD-derived radioactivity) in pheasant chicks to be 13 d, while that of adult hens was

determined to be 378 d. Pheasant chicks deplete inherited yolk reserves during the first few days after hatching, causing whole-body lipid content to decline. Based on this observation, it was suggested that high rates of TCDD elimination in chicks occur primarily via partitioning from tissues into contents of the gastrointestinal tract.

Modeling Exposure and Disposition of Contaminants in Oviparous Vertebrates

On an absorbed-dose basis, early life stages of oviparous vertebrates often exhibit greater sensitivity to chemical contaminants than do adult life stages. For example, lake trout fry exposed as eggs to TCDD exhibit a suite of toxic effects including pericardial and yolk sac edema, subcutaneous hemorrhaging, cranio-facial alterations, and arrested development. The concentration of TCDD in eggs required to elicit this response is between 40 and 80 pg/g, regardless of whether the compound is injected directly, taken up from contaminated water, or inherited via maternal transfer (Walker et al. 1994). In contrast, adult lake trout live apparently "normal" lives even when whole-body concentrations of TCDD approach 100 pg/g. These and similar observations underscore the importance of accurately assessing the exposure of early life stages.

One means of performing exposure assessments is through the development and use of mathematical models. These models formalize and simplify complex phenomena and can be used to extrapolate limited information. Research is conducted in support of model development and as a means of evaluating model performance. Descriptive research is frequently conducted in advance of more mechanistic work to define the system under study and to collect an empirical dataset, which then becomes the basis for developing mechanistic hypotheses. To date, modeling efforts with oviparous vertebrates have focused primarily on descriptions of chemical uptake and disposition in adult fish. Several different modeling approaches have been used, depending upon the specific application. These models vary considerably with respect to underlying assumptions and the level of mechanistic detail. All, however, are based on established principals of chemical mass-balance. Readers interested in more information on modeling efforts with adult fish are referred to several excellent reviews (e.g., Barron et al. 1990; McKim and Nichols 1994; Gobas and Morrison 1999).

Material presented earlier in this chapter indicates that many environmental contaminants are transferred from female fish or birds to their eggs. Several authors have suggested that this transfer provides a means by which adult females eliminate accumulated chemical residues. By comparison, the disposition of chemical within the developing embryo has received less attention. In the following sections, a novel approach is described for modeling in ovo exposures to lipophilic organic com-

pounds. Based on the principal of chemical fugacity, this approach is deliberately presented as a simple and generic method that can be used to derive a first-order assessment of chemical exposure. In subsequent sections, an effort is made to describe 2 kinetic models for contaminant bioaccumulation in birds. The first is a compartmental model for organic chemical accumulation in the herring gull; the second is a bioenergetics-based model for PCB uptake by nestling tree swallows. Together these 2 efforts represent the totality of exposure modeling efforts with birds published to date. A concluding section provides suggestions for future research, with an emphasis on the development of advanced kinetic models for birds.

Fugacity approach to describing in ovo exposure

The goal of an exposure assessment is to relate chemical concentrations in a range of environmental media (e.g., water, sediment, diet) to the effective concentration at the site of toxic action. It is well recognized that physicochemical characteristics of both environmental media and biological material (i.e., tissues) have a profound effect on the relationship between an external chemical concentration and that which exists within the organism. For example, the long-term exposure of a fish to 1 ng/L of pentachlorobenzene in water results in a whole-body concentration similar to that which is achieved when the fish is exposed to a much greater concentration (10,000 ng/kg) in the diet. Contaminant concentrations also can vary among different tissues as a result of differences in their chemical makeup.

The role of physicochemical factors in controlling exposure is very important for toxicological assessments. Environmental monitoring studies provide an indication of chemical exposure in terms of whole organism or tissue concentrations. The relevance of these concentration data for toxicological assessments may be unclear, however, because the chemical makeup of tissues that comprise the suspected target organ is different from that of tissues that were sampled. To avoid this problem, it is important to express concentrations in the ambient environment and inside the organisms in terms of "effective" concentrations. These effective concentrations are equivalent to the "chemical potential" or "chemical activity" used by chemists and engineers to express the thermodynamic status of the chemical. There are various ways to express, quantify and measure chemical activity in the ambient environment and the organism. One approach that has already been discussed is to express chemical residues in tissues as lipid-normalized values. As indicated previously, differences in concentrations of persistent organic chemicals among tissues often disappear when they are expressed on a lipid-normalized basis, indicating that a "common" concentration applies to these tissues (Geyer et al. 1985). In these cases, concentrations measured in one tissue can be interpreted in terms of the effective concentration in another tissue or organ.

A second technique is to use the principal of chemical fugacity to characterize the thermodynamic status of a system. Applications of the fugacity concept to environ-

mental modeling have been discussed by Mackay (1991). The second law of thermodynamics states that when a chemical is allowed to exchange between 2 adjoining media, it will tend to approach a situation wherein chemical fugacities in both media are the same. In an environmental setting, which contains media of differing chemical composition, this means that the diffusing chemical substance will tend toward equal fugacities but not equal concentrations. The main advantage of the fugacity approach is that physicochemical factors affecting relationships between external and internal chemical concentrations can be isolated from other factors because all concentrations are expressed on a common basis. The advantage to toxicologists is that fugacity can be directly related to chemical concentration at a target organ regardless of its composition. Developments in gas sparging (Yin and Hassett 1986; Sproule et al. 1991; Horstmann and McLachlan 1992) and solid phase extraction techniques (Zhang and Pawliszyn 1990; Zhang et al. 1994; Parkerton and Stone 1996) provide a very promising future for the measurement of chemical fugacities in environmental media and tissues.

A fugacity-based model for maternal deposition of hydrophobic organic contaminants into eggs

The primary assumption of the egg-deposition model for hydrophobic organic chemicals is that chemical transport from maternal tissues to developing eggs follows a set of passive (non-energy consuming) transport processes resulting in a thermodynamic equilibrium. This assumption is based on the observations that

- the internal distribution of hydrophobic organic chemicals within organisms tends to be relatively fast, often resulting in a tissue distribution that is homogenous when expressed on an appropriate basis (e.g., fugacity or lipid based concentration) (Clark et al. 1987; Nichols et al. 1990);
- hydrophobic organic chemicals generally exhibit high rates of permeability in biological membranes (Stein 1981);
- egg formation involves the transfer of lipoproteins from maternal tissues to the eggs; and
- the metabolic biotransformation of contaminants in eggs is often negligible because the necessary enzymes are not yet active.

Adopting this assumption, a chemical equilibrium can be formulated in terms of the chemical fugacities (Pa) in the maternal tissues (f_M) and the eggs (f_E): $f_E = f_M$. Chemical fugacity is equal to the ratio of the chemical concentration C (in mol/m^3) in a tissue and its fugacity capacity Z (mol/m^3 Pa): $f = C/Z$. It follows that chemical concentrations in the eggs (C_E) and the maternal tissues (C_M) reflect the ratio of the fugacity capacities of the eggs (Z_E) and the maternal tissues (Z_M), resulting in an egg-to-mother concentration factor (EMF) of:

$$EMF = C_E/C_M = Z_E/Z_M \qquad \text{Equation 2-1.}$$

Because a large number of studies have demonstrated that hydrophobic organic chemicals in organisms largely reside in lipids (Geyer et al. 1985) and that the solubility of contaminants does not differ substantially between different types of lipids (Dobbs and Williams 1983), it is reasonable to assume that Z_E and Z_L are approximately equal to $L_E Z_L$ and $L_M Z_M$ respectively, where L_E is the lipid content of the eggs, L_M is the maternal tissues, and Z_L is the fugacity capacity of both maternal and egg lipids. Substituting these relationships in Equation 2-1 gives us Equation 2-2:

$$EMF = C_E/C_M = L_E/L_M \qquad \text{Equation 2-2.}$$

Equation 2-2 suggests that the relationship between chemical concentrations in the egg and the maternal tissue simply reflects relative differences in lipid content. It follows, therefore, that if chemical concentrations are expressed on a lipid-weight basis as C_{EL} and C_{ML}, the relationship between the lipid based chemical concentrations in the eggs and the maternal tissues, which will be referred to as EMFL, is

$$EMFL = C_{EL}/C_{ML} = 1.0 \qquad \text{Equation 2-3.}$$

Equation 2-3 states that on a lipid-weight basis, chemical concentrations in eggs and maternal tissues will tend to approach equality. An important implication of this result is that the concentration of a chemical contaminant in an egg may be predicted from the concentration of the chemical in maternal tissues, provided that the lipid contents of the egg and maternal tissues are known.

It should be stressed that Equations 2-2 and 2-3 can be used to represent a chemical equilibrium only if 1) the fugacity capacity of different types of lipids is approximately the same and 2) tissue components other than the lipids do not contribute significantly to the total fugacity capacity of the eggs or the maternal tissues. If the lipid content is very low, the EMFL can be expected to differ from 1.0 even if a chemical equilibrium between eggs and maternal tissues exists. Also, the EMFL can be expected to differ from 1.0 while an equilibrium exists if there are significant differences in lipid composition between maternal tissues and eggs and in solubilities among different types of lipid in the eggs and maternal tissues. The error associated with the assumption that lipids are the sole component of egg and muscle tissue providing fugacity capacity for chemicals and that the fugacity capacity of different kinds of lipids are similar may contribute to the variability in observed EMFLs around the predicted model value of 1.0.

Model versus data

A considerable amount of research has been done on the transfer of chemical contaminants from maternal tissues into eggs of both birds and fish. For example, Braune and Norstrom (1989) published ratios of egg/female concentrations in herring gulls and suggested a mechanism for maternal transfer; Niimi (1983) provided data on the transfer of several contaminants into rainbow trout eggs; Miller (1993) examined differences in maternal transfer of organochlorines into

eggs of lake trout and chinook salmon; and Miller and Amrhien (1995) characterized the maternal transfer of PCBs and several chlorinated pesticides in Lake Superior siscowet, a subspecies of lake trout noted for its high lipid content.

A comprehensive collection of maternal transfer data was recently published by Russell et al. (1999). These investigators combined existing data with the results of field studies on Lake Erie to determine maternal transfer and in ovo bioaccumulation of 44 hydrophobic organic chemicals in 9 species of fish, one species of bird (herring gull), and 1 reptile species (snapping turtle, *Chelydra serpentina*) (Figure 2-4). An examination of these data suggests that there are very large differences among fish species in the egg/female lipid content ratio, ranging from approximately 0.78 for carp (*Cyprinus carpio*) to 41.9 for the freshwater drum (*Aplodinotus grunniens*). The egg/female concentration ratios (EMF) for test chemicals also varied considerably among species, ranging from 0.69 to 51.5. In terms of wet-weight-based concentrations, therefore, the eggs of some species appeared to receive a greater dose than others. However, when chemical concentrations in the eggs and

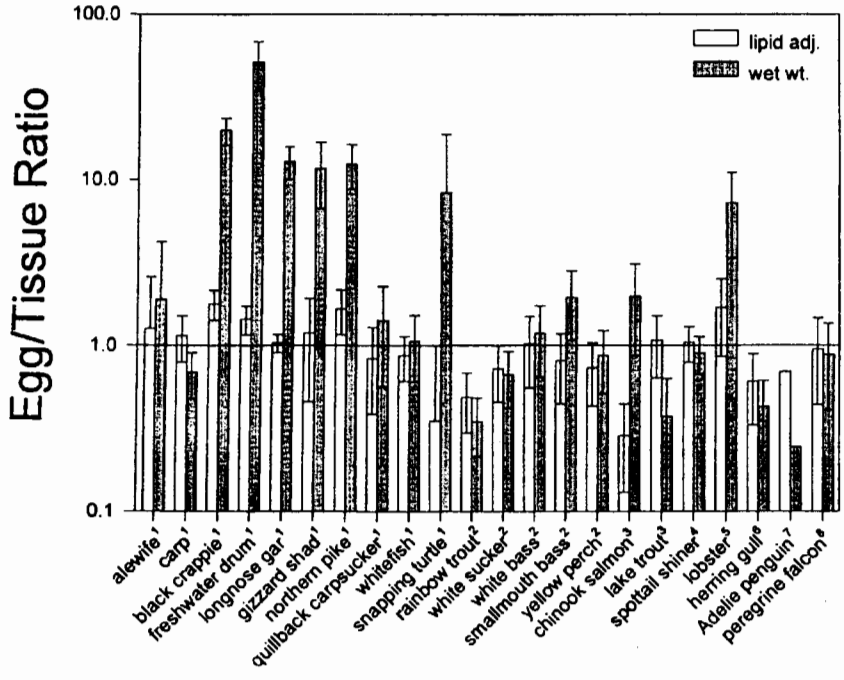


Figure 2-4 Observed relationships between chemical concentration in eggs and maternal tissues, as expressed by lipid-weight-based (gray bars) and wet-weight-based (white bars) egg/maternal tissue concentration ratios, in several classes of oviparous organisms. The solid horizontal line represents the chemical equilibrium model prediction for lipid-based concentration ratios. Data are from [1]Russell et al. (1999), [2]Niimi (1983), [3]Miller(1993), [4]Noguchi and Hesselberg (1991), [5]Guarino et al. (1974), [6]Braune and Norstrom (1989), [7]Tanabe et al. (1986), and [8]Cade et al. (1968).

the females were adjusted for lipid content, the egg/female concentration ratios (EMFL) were normally distributed with a mean of 1.22, a 2.5 percentile of 0.56, and a 97.5 percentile of 2.51. Importantly, EMF and EMFL values did not vary with chemical log K_{ow}, suggesting that maternal transfer in fish is essentially independent of chemical hydrophobicity. The mean EMFL for 24 chemicals in snapping turtles was 0.35. A statistical analysis suggested that this value was significantly less than 1.0 ($\alpha = 0.05$). The mean EMFL for 47 chemicals in herring gulls, as reported by Braune and Norstrom (1986), was 0.61. This value was also significantly lower than 1.0.

On average, therefore, eggs from snapping turtles and herring gulls did not contain as much chemical as the fugacity model would have predicted from chemical residues in female parents; at the same time, fish eggs contained chemical residues that were slightly higher than expected. Nevertheless, it is remarkable that concentrations in eggs and maternal tissues were so similar across such a large range of species and chemicals. This suggests that at the time of egg deposition, contaminant concentrations in the eggs and maternal tissues of fish, turtles, and birds are close to chemical equilibrium.

When all data were combined, the variability in the mean EMFL, expressed as 95% probability intervals, was approximately a factor of 2. This means that 95% of the EMFL values were found to be between 0.56 and 2.51. Part of this variability may originate from differences in lipid composition (not lipid content) between the egg and the mother. Differences in lipid composition may provide a somewhat different fugacity capacity to the egg and the mother if chemicals exhibit different solubilities and fugacity capacities in different types of lipids (e.g., phospholipids versus nonpolar triglycerides). In addition, tissue components other than lipids may have a small effect on the fugacity capacity of the egg contents. The lipid contents, as determined by the extraction techniques used in the various studies, may not account for the role of non-lipid components of the eggs and the female fish on the fugacity capacity of the chemicals in the eggs and the female fish, hence causing an apparent (but not real) deviation from a thermodynamic equilibrium. This apparent deviation may "disappear" if fugacities are measured directly rather than indirectly as lipid-based concentrations. If the observed uncertainty in the EMFL is applied to model predictions of egg exposure for new chemicals or species, it follows that lipid-based egg concentrations can be expected to fall within a range of 0.56 to 2.51 times the lipid-based concentration in the mother. Because all of the current observations regarding maternal transfer of organic chemicals involve persistent hydrophobic organic chemicals, it is unclear whether this simple partitioning model applies to less hydrophobic chemicals or to chemicals that undergo metabolic biotransformation. Nevertheless, the information for persistent chemicals allows for some extrapolation. If the chemical is metabolized, it is possible that the model does not apply. For example, if the chemical is preferentially metabolized in the eggs, the fugacity in the eggs can be expected to be lower than that of maternal tissues.

Preferential metabolism by the female may have little effect on model predictions if concentrations of the parent compound remain relatively constant. This is because the egg would "inherit" parent compound in the same manner as an unmetabolized compound; that is, chemical activity in the female and the egg would be reduced by metabolism to a similar extent.

Chemical exchange between deposited eggs and the environment

Maternal transfer is not the only process that affects the concentration and fugacity of chemicals in the eggs. Eggs deposited by fish may be exposed to contaminants in water and benthic sediments, while for reptiles there may be exposure to contaminants in soil and groundwater. Bird eggs may also experience some exposure to environmental contaminants, although the amount of chemical exchange that occurs may be limited by a relatively impermeable shell. Because of the multiple media involved in egg exposure, it is advantageous to characterize the potential for chemical transfer in terms of the differences in chemical fugacity in the eggs and those in the ambient environment. If the chemical fugacity in water and/or sediment exceeds the fugacity in the eggs, there is a potential for the concentration and fugacity of the chemical in the eggs to increase. This may occur, for example, when fish return from relatively clean sites (e.g., ocean or main river stem) to deposit their eggs in areas that are subject to contamination (e.g., gravel beds or sloughs). In these situations, the fugacity in the eggs will tend toward the prevailing fugacity in the environment. The extent to which ambient fugacity is achieved is dependent on the rate of diffusion of the chemical into the eggs and the duration over which the exposure occurs. These diffusion rates are largely unknown but can be expected to depend on hydrodynamic conditions (water flow over eggs), the thickness and composition of the chorion, the surface area and size of the eggs, and molecular characteristics related to diffusion (e.g., molecular volume). Although the relevant data do not exist, it is not inconceivable that chemical concentrations and fugacities in eggs can be substantially increased (perhaps approaching the ambient fugacity) under conditions when relatively uncontaminated eggs are exposed to high concentrations of chemicals at contaminated sites.

If the chemical fugacity in deposited eggs is less than that in the ambient environment, there is a potential for the chemical fugacity in the eggs to decrease. The rate of depuration of contaminants from the eggs is affected by the same diffusional and temporal factors that control the rate of uptake. The limited amount of data on chemical elimination from fish eggs was discussed previously. This elimination is expected to occur more slowly than that from yolk-sac fry and may be negligible for high log K_{ow} compounds even in a relatively clean environment. If significant depuration occurs, however, as can be expected for some lower K_{ow} chemicals, then the eggs may loose some of their chemical burden and approach the chemical fugacity in the environment.

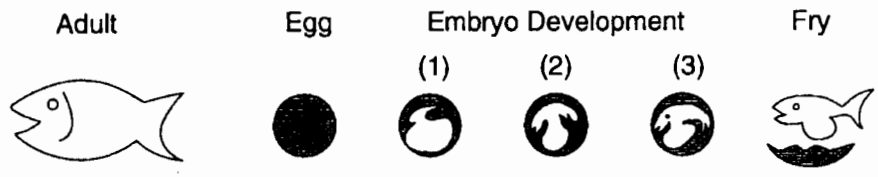
Finally, it is well known that some very hydrophobic organic chemicals ($\log K_{ow} > 6$) have a tendency to accumulate in aquatic food chains resulting in chemical fugacities that increase with each trophic level. Under these circumstances organisms at the highest trophic levels can be expected to deposit eggs that possess a chemical fugacity greater than that in surrounding water or sediment. Unless the eggs are being deposited in a location of greater contamination than that in which the female resided, there is a good possibility that chemical fugacity in the eggs is greater than that of the environment. Under these conditions, in ovo concentrations and fugacities of these chemicals could be expected to fall.

Chemical exposure during embryo development

During embryonic development, material that was transferred to the egg for nutritional purposes is consumed, changing the environment within which the embryo resides. With respect to the distribution of hydrophobic organic chemicals, the most important change is a potential decline in lipid content. For example, Peakall and Gilman (1979) reported a 2-fold decline in the lipid content of herring gull eggs during incubation. This means that although there may be no change in chemical mass or concentration in the egg (assuming, for simplicity, no exchange with the environment), the lipid-based concentration can be expected to increase. In terms of the fugacity theory, this is equivalent to a drop in the fugacity capacity of the egg. Assuming that the chemical concentration does not change, this will result in an increase in chemical fugacity, because fugacity is equal to the ratio of concentration (C) and the fugacity capacity (Z): $f = C/Z$. This increase in fugacity would then be associated with an increase in the chemical's effective concentration, as illustrated in Figure 2-5. Because it is a "closed" system, the potential for consumption of stored yolk to increase fugacity may be greatest in bird eggs. In fish eggs an increase in fugacity can potentially be offset by elimination to the environment (assuming that the fugacity of the environment is similar to or less than that of the egg when it was deposited). The final concentration, therefore, would represent the net result of these 2 competing processes.

Conclusions

There is a considerable body of evidence indicating that concentrations of hydrophobic organic chemicals in eggs closely reflect the concentration in maternal tissues as long as the concentrations are expressed on a lipid-weight basis. The available data are reasonably consistent with a simple model that assumes that chemical concentrations in eggs and maternal tissues achieve a chemical equilibrium. The data and the model imply that chemical transfer into eggs is a passive process that follows fugacity gradients. The result is that the chemical fugacity in the egg is close to that in the maternal tissues. This simple equilibrium model can be used to provide first-order estimates of in ovo chemical exposure at the time of egg deposition based upon knowledge of maternal tissue residues, provided that the lipid contents of the maternal tissues and egg are known.



	Adult	Egg	Embryo (1)	Embryo (2)	Embryo (3)	Fry
f (Pa):	1	1	1.1	1.3	2	4
Z ($^{mol}/_{m^3}$Pa):	10	20	18	15	10	5
Z_L ($^{mol}/_{m^3}$Pa):	100	100	100	100	100	100
C ($^{mol}/_{m^3}$):	10	20	20	20	20	20
C_L ($^{mol}/_{m^3}$):	100	100	110	130	200	400

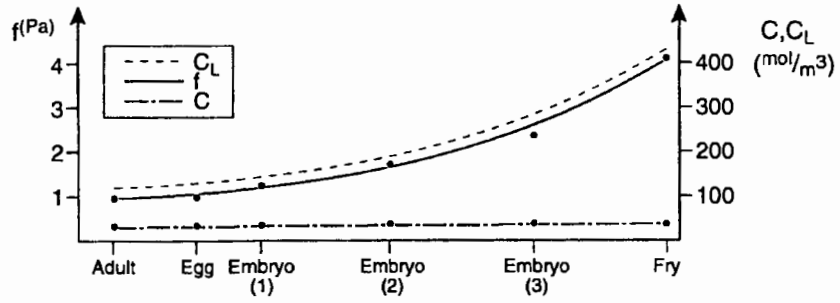


Figure 2-5 Illustrative example of the expected effect of in ovo embryonic development on the wet-weight-based chemical concentration in the egg (C), the lipid-based chemical concentration in the egg (C_L), the chemical fugacity capacity of the egg (Z) and the chemical fugacity (f) in the egg.

During embryo development, the lipid-based chemical concentration has the potential to increase, particularly within bird eggs, resulting in an effective concentration that is greater than that of the mother. If the embryo exhibits the same toxicological sensitivity to the contaminant as the mother, the expected increase in effective chemical concentration in the egg will cause a degree of toxicological risk to the embryo that is greater than that to the mother. An increase in the sensitivity of the embryo relative to that of the mother would tend to make this situation even worse.

Chemical uptake and accumulation models for birds

The foregoing discussion highlights the fact that in many instances it is possible to describe chemical uptake and bioaccumulation in oviparous vertebrates using an equilibrium partitioning approach (i.e., by assuming that an organism is in thermodynamic equilibrium with all or part of its environment). Alternatively, a dynamic equilibrium may occur as the result of a balance between uptake and elimination, including metabolic biotransformation. In other cases, however, chemicals do not

attain chemical equilibrium, either within an organism or between an organism and its environment. Factors that tend to promote this condition include fluctuating exposures, rapid growth, rapid mobilization of lipid stores, and changing metabolic capabilities, particularly when these factors are combined with chemical attributes (e.g., hydrophobicity, low bioavailability) that result in low rates of uptake and elimination. An examination of field residue data suggests that as a generalization, the potential for chemical disequilibrium between an organism and its environment increases at progressively higher trophic levels (Clark T et al. 1988). Thus, while an equilibrium may exist between a female and its eggs, the female may not be in equilibrium with its environment.

Kinetic processes can be included in fugacity-based models, which then combine information on partitioning, transport, and biotransformation (Mackay 1991). The advantage of these models is that they distinguish between kinetic and physico-chemical factors controlling chemical distribution, which improves insights into the chemical distribution process. A more "classical," compartmental kinetic modeling approach may also yield insights, particularly when fitted rate constants are interpreted in the context of relevant physiological information (e.g., glomerular filtration rate for a compound eliminated in urine). Physiologically based toxicokinetic (PBTK) models are based on anatomical, physiological, and biochemical information, and they do not require a priori collection of data to fit the value of kinetic rate constants. In practice, however, they incorporate many of the same principals employed in fugacity-based modeling, insofar as mass-balance equations for individual tissues require the specification of chemical capacity relative to that of blood. For many compounds this chemical capacity is determined largely by tissue lipid content.

The following sections describe 2 models that have been developed to describe contaminant uptake and accumulation by birds. The first is a 2-compartment kinetic model for herring gulls published by Clark et al. (1987). Although originally formulated with forward and reverse rate constants for internal chemical distribution, implementation of the model was subsequently simplified by assuming that the 2 compartments were in chemical equilibrium. The second model is a bioenergetics-based model for tree swallows given by Nichols et al. (1995). This model is notable because it yields concentration predictions based upon continuously changing rates of chemical uptake and organism growth.

Two-compartment, open model for herring gulls

The herring gull is the only year-round, resident piscivorous bird species in the Great Lakes region. In the 1960s it was found that gull populations in Lakes Ontario and Erie were declining and that embryo mortality was the principal cause (Keith 1966; Ludwig and Tomoff 1966). Efforts to identify the compounds responsible for this mortality and the mechanisms by which they act were complicated by the fact that gulls are simultaneously exposed to many bioaccumulative contaminants,

including pesticides, PCBs, dioxins, and methylmercury. Herring gulls are relatively resistant to eggshell thinning by DDE (Gilbertson 1974; Gilman et al. 1977). Perhaps the strongest case to date has been made for compounds with a TCDD-like mode of action, resulting in a suite of toxic effects that include porphyria, wasting syndrome, and edema (reviewed by Gilbertson 1991; Giesy et al. 1994; Chapter 4 of this volume).

Efforts were initiated in the late 1960s to monitor contaminant levels in herring gull eggs (Mineau et al. 1984). These efforts continue to this day and have provided detailed descriptions of geographical and temporal trends in organochlorine contaminants in eggs collected from each of the Great Lakes (Weseloh et al. 1990, 1994; Ewins et al. 1992; Hebert et al. 1994). Organochlorine levels in eggs declined substantially in the 1970s, coinciding with improvements in herring gull reproduction. Efforts to understand these changes in relationship to contaminant levels in environmental media and adult birds were complicated, however, by the long-lived nature of gulls and by seasonal changes in locality, dietary choice, and lipid status. Studies were therefore initiated to better characterize contaminant uptake, distribution, and clearance in both captive and free-living gulls, maternal transfer of chemical residues to eggs (Anderson and Hickey 1976; Norstrom, Clark, Jeffrey et al. 1986), and basic aspects of gull biology and bioenergetics (Norstrom, Clark, Kearney et al. 1986).

These studies eventually led to the development of a 2-compartment open model for gulls (Figure 2-6; Clark et al. 1987), with elimination from a central "plasma" compartment and chemical exchange between plasma and a peripheral "fat" compartment. Employing symbols used by the authors, the contaminant concentration in plasma is C_p, contaminant burden in fat is X_f, and the weight of the fat pool is W_f. The fat compartment was allowed to change in size as a means of describing seasonal changes in whole-body lipid content. First-order rate constants for chemical transport into and out of fat are k_{pf} and k_{fp}, respectively. The first-order rate constant for clearance by all mechanisms is k_{pc}. A simplification of this model was also achieved by assuming that the distribution of chemical between fat and plasma occurs rapidly such that the ratio of distribution rate constants can be represented as a "partition coefficient" K_{pf}. With this simplification, the final equation for calculation of plasma concentration becomes: $C_p = X_f/W_f) C K_{pf}$.

Data collected from dosing studies with 10 different compounds were used to estimate the values of all relevant model parameters. The model was then evaluated by comparing simulated chemical residues with measured values from a second group of dosed animals (Figure 2-7). Model performance was very good, as might have been expected, since a similar protocol was used for both calibration and evaluation. Nevertheless, the model performed better for some compounds than others, suggesting in several cases that basic modeling assumptions had been violated (principally the assumption of instantaneous uptake of the administered dose). The value of the gull model as an aid to interpreting chemical residues was

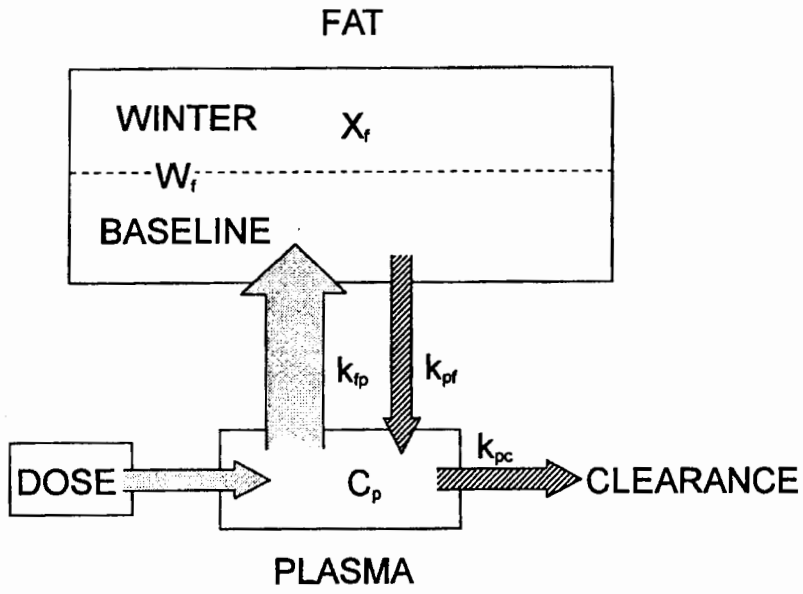


Figure 2-6 2-compartment, open model for contaminant toxicokinetics in the herring gull (adapted from Clark et al. 1987)

demonstrated by simulating tissue-residue concentrations under a variety of exposure scenarios. In this manner, it was possible to show that the time-constant for reestablishing an annualized steady-state concentration after a hypothetical change in food concentration was as much as 3 years for slow-clearing compounds like mirex (Clark T et al. 1988).

Bioenergetics-based model for tree swallows

As with herring gulls, a model for swallows was developed to aid in the interpretation of measured tissue residues (Nichols et al. 1995). Tree swallows have attracted special attention because they feed extensively on emergent aquatic insects. Persistent organic compounds that are present in sediments tend to accumulate in larval forms of these insects, often approaching an equilibrium distribution, as indicated by BSAFs (lipid and carbon normalized) of approximately 1.0 (Ankley et al. 1992). Upon emergence as adults, these insects provide a vector for translocation of sediment contaminants to insectivorous birds (Larsson 1984; Clements and Kawatski 1984; Gobas et al. 1989; Kovats and Ciborowski 1989; Fairchild et al. 1992). The tree swallow is a widely distributed passerine species. Throughout their breeding range, population densities are limited by the availability of nesting cavities. By placing nest boxes in suitable locations it is possible to create a study population of swallows, often within the first year. In contrast to adults, which may accumulate chemical residues while overwintering, nestling swallows are exposed primarily to compounds present around the nest site, combined with those inher-

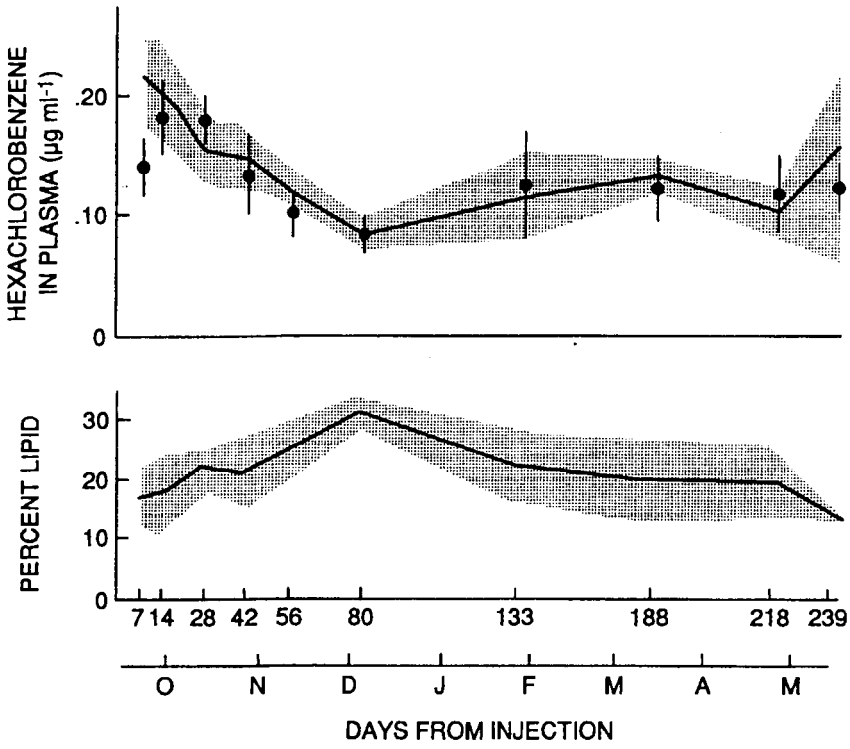


Figure 2-7 Hexachlorobenzene concentrations in gull plasma following a single i.p. injection (adapted from Clark et al. 1987). The model simulation is shown as a solid line, measured values are given as individual points.

ited as egg residues. For this reason, nestling tree swallows have been proposed for use as sentinels of local sediment contamination.

A bioenergetics-based modeling approach was used to simulate chemical accumulation by swallows because of the need to explicitly describe both dietary uptake of contaminant and changing growth rate during the nestling development period. Contaminant concentrations in nestling tree swallows have been observed to decline for a time after hatching. Termed "growth dilution," this result can occur only if the efficiency with which ingested energy is converted to tissue mass exceeds the assimilation efficiency for the toxicant. Later, as nestlings become more active and start to thermoregulate, the proportion of ingested energy allocated to growth declines and contaminant levels increase. Although varying in detail, this pattern appears to be a general one and has been reported for nestlings of both precocial and altricial species (Robinson et al. 1967; Persson 1971; Anderson and Hickey 1976; Harris et al. 1993; Ankley et al. 1993).

A bioenergetics-based model defines a system in terms of energy fluxes. A common form of such a model is to define the growth of an individual organism as the net result of an energy-mass balance. This is accomplished by using conversion factors to transform biomass into energy equivalents, utilizing the calorie scale of measurement. Thus, the "energy density" of a whole organism, individual tissues, or prey item can be defined in calories per gram, as determined using bomb calorimetry or other techniques. Methods also have been developed to estimate energy expenditures for physiological "housekeeping" functions, such as basal metabolism, digestion, and waste processing, as well as energy requiring activities such as prey capture, migration, thermoregulation, and mating. Excellent reviews are available on the application of this modeling approach to fish (Kitchell 1983; Adams and Breck 1990; Hansen et al. 1993; Lucas 1996) and birds (Kendeigh et al. 1977; Whittow 1986).

Typically, the energy-mass balance on an individual organism is solved for the value of the production term:

$$P = C - (F + U + R) \qquad \text{Equation 2-4,}$$

where P = tissues formed, C = food consumed, F = fecal losses, U = urinary losses, and R = respiration. All terms in the equation appear as rates. Loss terms are commonly summed and subtracted from consumption. Integrating the equation transforms production into mass as total calorie equivalents. Mass expressed as grams of animal is obtained by multiplying calories by the appropriate energy density function.

Simulations for tree swallows were obtained using PCB concentrations in insects and eggs as inputs to the model. The model reproduced observed rates of growth and the reported pattern of PCB growth dilution. The predicted contribution of maternally derived residues to total residues at fledging ranged from 10 to 25% and varied inversely with the extent of site contamination. PCB concentrations in birds from a relatively uncontaminated reference site were well described, but residues from an area of known sediment contamination were overestimated (Figure 2-8). An examination of the levels of individual congeners suggested that this result was not due to differences in metabolic biotransformation. Instead, it was suggested that there may have been differences in dietary composition among sites or a general underestimation of prey consumption rate.

Future research needs

Data needs for model development

To further explore the mechanism and the applicability of fugacity-based models of in ovo exposure of embryos to contaminants, a number of suggestions regarding research and data needs can be made. One of the key assumptions in the fugacity-based, maternal transfer model presented in this chapter is that the fugacity capacity of eggs and maternal tissues for hydrophobic organic chemicals is exclu-

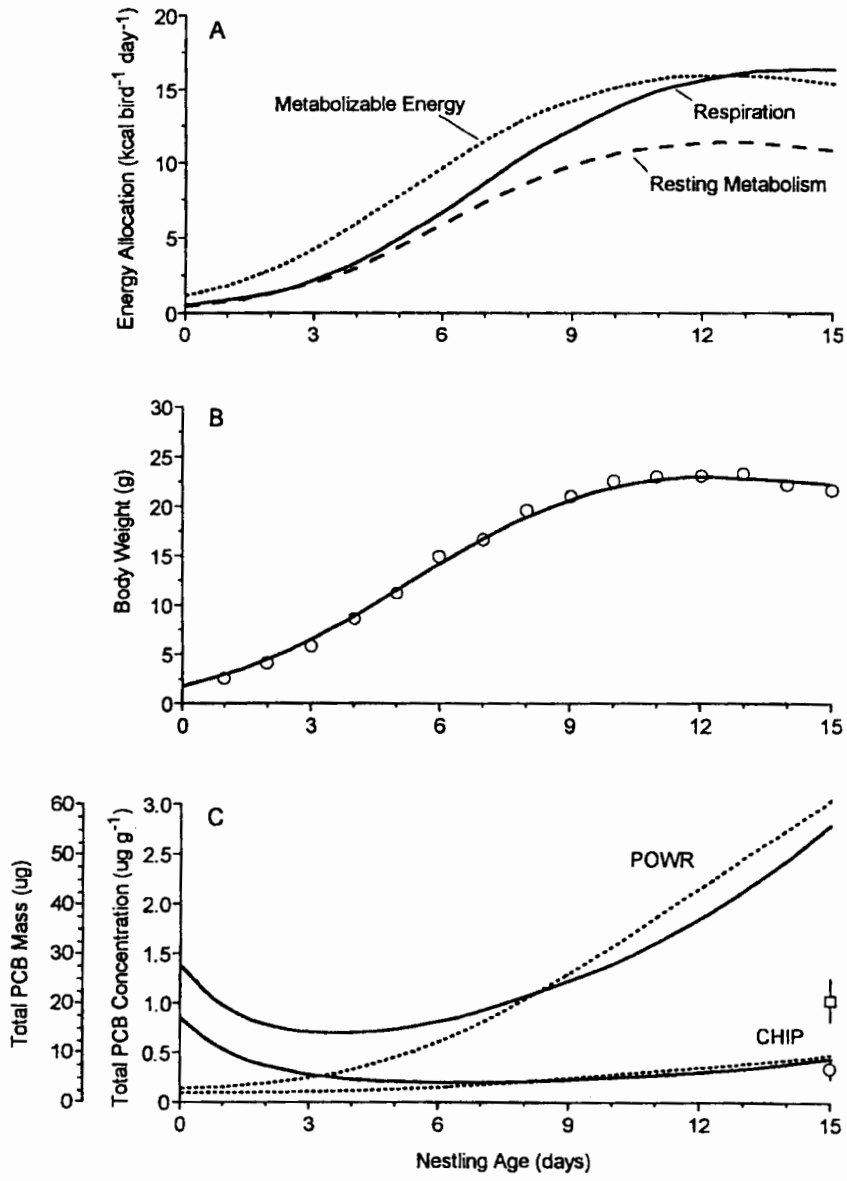


Figure 2-8 A) Energy budget predicted by a tree swallow bioenergetics model. Simulations correspond to model predictions of metabolizable energy (dotted line), total respiration (solid line), and resting metabolic rate (dashed line). B) Growth of nestling tree swallows from the Saginaw River watershed, Michigan. Measured values are shown as individual points. C) Predicted and observed total PCB residues in nesting tree swallows from an unpolluted upstream site (CHIP) and a polluted downstream site (POWR) on the Saginaw River, Michigan. The mean concentration of total PCBs measured in 15-d old nestlings is shown as an open circle (CHIP) or open square (POWR). Model simulations are shown as solid (PCB concentration) and dashed (PCB mass) lines. Reprinted with permission from Nichols et al. 1995. Copyright 1995 American Chemical Society.

sively the result of the amount of lipids in the eggs and maternal tissues. This ignores the contribution of non-lipid components of the eggs and tissues (e.g., proteins, glycogen) to the fugacity capacity. To further test the assumption of equilibrium partitioning as a mechanism of maternal transfer into eggs, it is important to first develop methods for determining fugacities and the fugacity capacity of contaminants in eggs and maternal tissues and then to apply these methods to investigate fugacity gradients between maternal tissues and eggs.

While this chapter focuses on maternal transfer and initial in ovo exposure of eggs to contaminants, it is important to realize that there may be toxicologically significant changes in the effective concentration of a compound during in ovo embryo development simply because of changes in physicochemical composition of the embryo. Fugacity analysis of contaminants during embryo development is probably one of the better methods to investigate changes in these effective concentrations. Understanding the effective exposure during embryo development also plays a role in determining the sensitivity of early life stages relative to adult life stages. Finally, while maternal transfer is an important mechanism controlling in ovo exposure of embryos to contaminants, it is not the only one. Chemical exchange between the embryo and its ambient environment can play an important role as well. Currently, it is difficult to assess the exposure of fish embryos under actual environmental conditions because trans-chorionic diffusion rates are not commonly known.

The scientific information needed to develop kinetic models for birds consists broadly of mechanistic, or "process," information and high-quality kinetic data. Among the many factors that have potential to impact chemical uptake and disposition, there is a particular need to understand the biological and physicochemical determinants of oral bioavailability. Modeling efforts with fish have shown that differences in dietary uptake efficiency can contribute substantially to differences in bioaccumulation rate among chemicals, species, and life stage. Limited modeling efforts with birds have led to the same conclusion (Nichols et al. 1995).

An improved understanding of metabolic biotransformation is also critical. Although it is unreasonable to expect that detailed information can be collected for all species of interest, it may be possible to generalize activity and specificity information to taxonomic subgroups. The consideration of compounds that undergo metabolic biotransformation also requires improved methods for metabolite identification and quantitation. Most of the metabolism information collected to date has been obtained using in vitro systems. A need exists for improved methods to monitor the kinetics of labile metabolites in vivo. Such information could be used to investigate metabolite disposition within exposed animals and would make possible direct in vitro/in vivo comparisons.

High-quality kinetic data is needed to relate the chemical time-course in whole-animal dosing studies or environmental exposures to the dosages given directly to eggs in the laboratory. This will require additional information on the biochemistry

and physiology of yolk deposition in eggs, with particular emphasis on the sources and fate of lipid and lipoprotein. One approach that could be used to address these questions would be to conduct a chemometric analysis of chemical residues in eggs, maternal parents (liver and fat), and dietary sources, thereby quantifying the "resemblance" of chemical residue profiles.

Finally, there is a general need to obtain detailed kinetic information for specific tissues and organs that can be linked in turn to mechanistic studies of effect. PBTK models are particularly well suited for this purpose. Information that would be required to develop a physiological model for birds is described below. Additionally, it is proposed that physiological models for birds could be combined with bioenergetics and natural history information to obtain chemical time-course predictions for individual tissues in realistic environmental exposures.

Development of physiologically based toxicokinetic models for birds

Physiologically based toxicokinetic models are developed from the physiology, biochemistry, and anatomy of the exposed organism and, as a result, can be used to predict chemical uptake and disposition without the prior need for dosing information. In a typical model, the animal is divided up into several compartments representing tissues of kinetic as well as toxicological significance. Chemical distribution among tissues is assumed to occur only via circulation. Thus, for each tissue group, the following information is required: 1) tissue volume, 2) arterial blood flow as a percentage of cardiac output, 3) equilibrium chemical partitioning between the tissues and blood, and 4) biotransformation rate and capacity parameters. In addition, mathematical descriptions are required for chemical flux across relevant exchange surfaces. Mass-balance expressions are written for each compartment and are solved simultaneously by numerical integration to obtain a solution set for each time point. A schematic illustration of a PBTK model is given in Figure 2-9. The symbols used in this figure refer to the following modeled quantities: Q_{alv} – ventiliation volume; C_{inh} – chemical concentration in inspired air; Q_c – cardiac output; C_{ven} – chemical concentration in mixed venous blood; C_{art} – chemical concentration in arterial blood; Q_f, Q_m, Q_r, Q_l, Q_s – blood flows to the fat, muscle, richly perfused, liver, and skin tissue groups, respectively; C_{vf}, C_{vm}, C_{vr}, C_{vl}, C_{vs} - chemical concentrations in blood exiting the fat, muscle, richly profused, liver, and skin tissue, respectively; K_m, V_{max} – rate and capacity parameters for saturable metabolic biotransformation. Modeling assumptions and the structural details that follow from them have been extensively reviewed (Gerlowski and Jain 1983; Rowland 1985; McKim and Nichols 1994).

The principal advantage of a PBTK model is that it yields predictions of the chemical time-course in specific tissues of interest. Moreover, because these models have biological and physicochemical integrity, they can be used to extrapolate predictions well beyond the range of experimental conditions. In practice, the development of such models follows an iterative process of simulation and data collection.

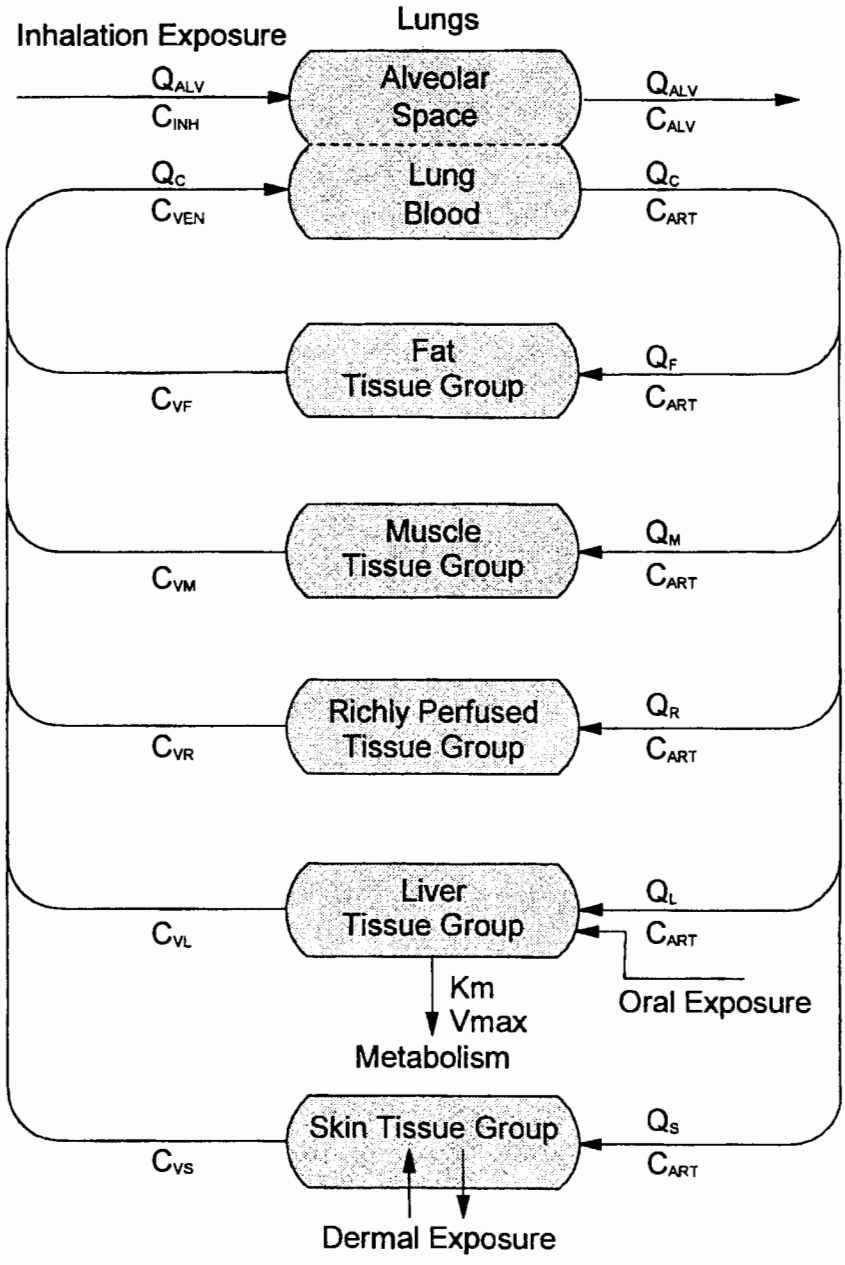


Figure 2-9 Schematic representation of a physiologically based toxicokinetic model for an air-breathing vertebrate

As experience is gained, sensitivity analyses are performed to assess the relative contribution of individual parameters to model performance. This information can then be considered along with the modeler's confidence in the various parameters to determine whether and how the model should be amended and which of the parameters requires further investigation.

PBTK models have been developed for more than 100 chemicals and for a dozen or more mammalian species, including man, and presently are being used in human-health risk assessment (Rietz et al. 1996). PBTK models have also been developed for fish, and have been employed to extrapolate kinetic information among species (McKim and Nichols 1994), evaluate metabolic rate and capacity parameters (Law et al. 1991), and investigate maternal transfer of hydrophobic organic compounds (Nichols et al. 1998). To date, there have been no attempts to develop PBTK models for birds.

Use of bioenergetics-based models to simulate the combined effects of chemical and non-chemical stressors on reproduction

It is important to recognize that avian reproduction and development can be impacted by non-chemical as well as chemical stressors. Perhaps the best example is that of habitat alteration, with associated impacts on food-web dynamics and the availability of suitable nesting sites. A potential consequence of these non-chemical impacts is a reduction in energy available for production of young and the growth of neonates. Numerous studies have shown that clutch size, egg quality, number of nesting attempts, and survival of young vary with the amount and quality of available food (Hepp et al. 1987; Esler and Grand 1994). These impacts have the potential to complicate the assessment of toxic effects. For example, the combined effect of DDE and a small reduction in ration on the reproductive performance of turtle doves (*Streptopelia risoria*) was much greater than the effect of either treatment by itself (Keith and Mitchell 1993). As demonstrated previously, bioenergetics-based models can be used to simulate dietary uptake of chemical contaminants on a whole-organism basis. In theory, this approach could also be used to describe chemical and non-chemical impacts on growth through their effects on organism energetics.

Bioenergetics models that explicitly describe reproduction can be developed by expanding the balanced energy equation as appropriate for the adult or offspring. For the adult (male or female), the respiration term is expanded to include energy expenditures for breeding behavior and parental care. The production term for the female is also expanded to include growth of both the parent and gametes. Summing these energy expenditures gives the total reproductive effort. For the offspring, respiratory expenditures may change dramatically due to increases in activity associated with fledging and the onset of thermoregulation. A period of weight loss frequently occurs as the energy demands of the young come to exceed the foraging capability of the parents. The data needs of this approach include both bioenergetics

and natural history information. Fortunately, there is a great deal of this information in the literature, due to long-standing interest in avian biology.

Linked bioenergetics and physiologically based toxicokinetic models

Finally, it should be possible to link both bioenergetics and PBTK models. Bioenergetics-based models describe organism growth, while PBTK models are generally formulated to calculate tissue volumes as fractions of total body weight. Individual tissues therefore change in size along with the organism. In some cases, such as the growth of gametes, tissue volume as a percent of body size changes in time. The growth of these tissues must be modeled explicitly and appropriate adjustments must be made to other tissue volumes. Physiological functions in both modeling approaches are usually scaled to body weight using allometric relationships.

The critical point of contact between these modeling approaches is provided by the dietary route of exposure. Bioenergetics-based models, combined with appropriate natural history information, describe what and how much an organism consumes. Knowledge of chemical residues in prey provides the delivered dose. PBTK models then translate this delivered dose into a tissue-level dose that can then be interpreted in the context of toxic effects. As indicated previously, respiration and growth terms in a bioenergetics-based model can be subdivided to account for energy expenditures associated with reproductive effort. Output from both types of models can be structured as input to population-level models. Thus, the production and growth of gametes and young can be expressed as measures of survival, fecundity, and recruitment.

Summary

Exposure of early life stages to xenobiotics is dependent upon a complexity of issues. Chemical release in areas of potential impact is an obvious prerequisite for exposure. Equally as critical are parental, early life stage, and environmental features that modulate exposure. Bioaccessibility (organismic exposure as modified by external environmental determinants) and bioavailability (systemic organismic exposure as defined by target organism determinants) categorically scale the toxicological importance of xenobiotics in the environment. Clearly, oviparous vertebrates are a special case for the early-life-stage exposure paradigm. There is the potential for not only direct environmental exposure with the accompanying modulating factors, but also for maternal transfer with its own array of determinants. With maternal transfer, historical residues of parental exposure are presented to early life stages as a concentrated source of contaminants within transferred nutrients. The existing literature, biased by the limited number of classes of chemicals examined, suggests that the compounds that show the greatest propensity to be transferred to early life stages are those that are more lipophilic, correlating in part to the characteristics of the transferred nutrients. Although conclusive evidence for the role of the yolk

lipoprotein vitellogenin in xenobiotic transfer is lacking for oviparous vertebrates, a number of studies have identified the presence of contaminants in the yolk and correlated the onset of vitellogenesis with the delivery of pollutants to the ovary. Lipids and lipid transfer dynamics from maternal sources to the developing ovaries do appear to be the critical determinants in the maternal transfer of lipophilic contaminants. Issues such as maternal concentrations of contaminants, the percent of total maternal lipid transferred to the eggs, egg weight as a percentage of the total maternal weight, and total maternal lipid burden appear to relate to the percent of whole-body contaminants that are transferred to eggs. Initial indications suggest reproductive life histories of the animals, different life strategies for resource mobilization for ovarian development, and dietary energetics may play an important role in determining the source and amount of the lipids and thus contaminants transferred by maternal transfer to early life stages. In birds, unique features such as homoeothermy and, in some cases, energetically expensive migration, courtship, breeding, and parental caregiving behaviors provide for unusual xenobiotic uptake and mobilization paradigms within this contextual framework. In totality, this information suggests that the deposition of contaminants in the egg mass by lipid association may be linked directly with species specific characteristics, maternal nutrient status, and the maternal resident xenobiotic body burden.

Available systemically upon mobilization of nutrient stores, maternally derived contaminants are present during critical phases of early development. Embryonic contaminant stores are mobilized at a time when there is an isolation of early life stages from parental assistance with regard to biotransformation and elimination. Limitations of like systems during early ontogeny put developing oviparous organisms at risk. For all practical purposes, the same mechanisms that provide (e.g., maternal nutrients) and protect (e.g., chorion, shell) during early development put early life stages of oviparous vertebrates in jeopardy when they are challenged by a chemical insult, especially those originating internally. For fish, elimination rates from embryos and yolk-sac larvae are generally much lower than the feeding post-yolk-sac stage which in turn is slower than more advanced stages. Changes in xenobiotic disposition with development, while of unknown etiology, are probably caused by development of uptake and depuration pathways, loss of contaminant storage areas in the form of yolk loss, and increased competency of developing metabolic systems. Generally, it is accepted that a substantial portion of apparent losses with development are also a result of growth dilution rather than of actual elimination. Phase I xenobiotic biotransformation, when measured under in vitro conditions, may occur in both the embryo and the larval stages of fish. P450 activities are generally higher in larval stages than in embryos, and activities for both are much lower than in adults. Fish egg and larval P450 activities may be induced by environmental routes, by experimental routes (e.g., by injection), and by maternal routes. While CYP1A is inducible pre- and post-hatch in fish, the induction response is greater after hatching. The reasons for differences in biotransforma-

tion and the induction response between life stages are unknown; however, they could be related to dosimetry considerations or variation in the responsiveness of the Ah receptor. A number of conjugation reactions have been identified for early life stages of fish. Although this is true, relatively little is known with regard to Phase II activities, induction, and ontogeny. Compositely, in vivo studies with early life stages have been inconsistent in demonstrating biotransformation. Small body size of fish in early life stages, whole-body and water dilution of metabolites, analytic sensitivity, differential metabolite solubility, and apparently low basal-uninduced metabolic rates complicate assessment of biotransformation. In general, more work will have to be performed on kinetic and biotransformational issues before the determinant factors and relative importance of real-world early-life-stage biotransformation in fish can be ascertained.

The importance of egg laying as a route of elimination for adult birds and as a route of exposure for early life stages appears to be species-specific and dependent on factors including egg and clutch size, maternal fat reserves, and the extent to which the reserves are mobilized. In terms of biotransformation, birds present a somewhat different picture than their piscine counterparts. P450 biotransformation varies greatly between adult birds of varying species, largely dependent on feeding ecology. P450 activities in birds are lower relative to mammals but higher than generally exhibited by other oviparous species. In contrast to mammals and other oviparous species, high levels of MFO activity are present during the embryonic and neonatal periods, suggesting that developing birds may be more independent in a biotransformational sense. The activity of these enzymes appears to be inducible by a wide variety of compounds, and the extent of this induction has been correlated with contaminant residues in field-collected eggs. Chemical kinetic data in neonates and juvenile birds are extremely limited. It is difficult, therefore, to assess the relative importance of growth dilution, metabolic biotransformation, and other routes of elimination in determining chemical residues. The oral bioavailability of most chemical classes in birds of any age remains essentially unknown.

Exposure and fate are critical determinants of chemical toxicity in early life stages of oviparous vertebrates. Clearly, the processes involved in direct exposure, maternal transfer, xenobiotic mobilization, biotransformation and excretory ontogeny are complex and interrelated with regard to their role in early-life-stage toxicity. The development and use of mathematical models provides a means to simplify complex phenomena and make predictive assessments. Thus far, most of the chemical modeling efforts with oviparous vertebrates are oriented toward the adult. Simplistic generic modeling approaches such as chemical fugacity show promise in the first tier assessment of chemical exposure for early life stages. Further understanding and better predictability are likely with model development incorporating physiological and bioenergetic-linked approaches.

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