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Adrenal Toxicology in Birds: Environmental Contaminants and the Avian Response to Stress

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BIRDS AS SENTINEL SPECIES

Wildlife species show great potential as sentinels for the early detection of adverse health effects of chemicals present in the environment, and thus as potential protectors of human health (Fox, 2001; Sheffield *et al*., 1998). A common definition for a sentinel species is any life-being (prokaryotic or eukaryotic, natural or transgenic, plant or animal, feral or domesticated) that can be used as an indicator of exposure to or toxicity from environmental contaminants and, therefore, can help to assess potential impacts on similar organisms, on populations or on ecosystems (Lower and Kendall, 1992; Stahl, 1997). The concept is important in the environmental health sciences because sentinel species can provide integrated and relevant information on the types, amounts, availability, and effects of environmental contaminants. Therefore, we can consider, simplistically, that sentinels are signaling potential environmental hazards (Frame and Dickerson, 2006; LeBlanc, 1995).

To be a sentinel, the species should be sensitive to the contaminant or contaminants of interest and, preferentially, have a wide geographical distribution, allowing the investigator to compare the response among individuals of the same species from multiple sites. Another consideration is the species' home range. Migratory or wide-ranging species normally convey the problem of a difficult

determination of the place and moment when exposure occurred. Therefore, it is generally advisable to select territorial, nonmigratory species with a restricted home range. If the contaminant is biomagnified, the selection of a species that is on a higher trophic level is justified. However, predatory vertebrates such as hawks, eagles, owls, mink, seals, and alligators are frequently protected or sparsed over a targeted site of interest. Although protected and/or endangered species can still be used if nonlethal sampling methods are used, a scarce sample will make it difficult to obtain statistically valid results (Frame and Dickerson, 2006).

Most of the previous requisites are so far achieved by many avian species (Becker, 2003). Moreover, birds are conspicuous organisms and relatively easy to observe. The general biology, behavior, and ecology of birds is normally well known compared to other vertebrates, which enhances their usefulness as sentinels by reducing the risk of misinterpretations. Birds occupy different positions in the food chain with numerous species in the higher trophic levels, allowing assessment of chemical contamination in several compartments of the ecosystems as well as biomagnification of persistent chemicals. Blood collection is relatively easy, and numerous nondestructive sampling techniques for other tissues and substrates are currently available, avoiding harming the study specimens, which is always advisable and a necessity when working with protected or endangered species. Samples such as feathers, feces, or eggs reduce the sampling effort and are easy to collect. Furthermore, birds have the advantage compared to many other vertebrates that it is normally easy to gather information on demographic parameters, such as population size and reproductive success. Colonial species allow the collection of samples and data in relatively short time (Kushlan, 1993). Also important, compared to other taxa, birds possess unique aspects such as a high metabolic rate, and on a mass-size basis often have higher metabolism and food consumption rates than, for example, placental mammals of similar size (WHO/IPCS., 0000). These factors, together with increased rates of metabolic biotransformation of xenobiotics, may contribute to an increased exposure to environmental contaminants. Migration, courtship, breeding, and parental behaviors require high-energy expenditure and are often accompanied by periods of starvation. Birds respond to these situations by storing and mobilizing fat depots, thereby raising the potential of increased exposure to lipophilic contaminants that are subsequently released from the lipid-rich tissues where they have been accumulated.

Provided the advantages mentioned above, there are also drawbacks and limitations for the use of birds as sentinels that may deserve attention depending on the aim of study (Becker, 2003; Furness, 1993). For example, the longevity of birds, although can be seen as an advantage because a long lifespan implies that the individuals integrate the effects of environmental stress over time, it also makes more difficult to establish the effects of a short-term perturbation. Similarly, the mobility of birds implies an integrative value of bioindication over broad spatial scales, but can hinder their site-specific use as indicators. For example, the

sympatric occurrence of different populations of a given species during migration or staging at one site may obscure local sources of environmental stress and reduce their value as indicators. Bird numbers are regulated by density-dependent processes, and so their population sizes may be somewhat buffered against the impacts of environmental changes. Because a multitude of variables affect demographic parameters and stages, the effects of specific factors can be difficult to isolate. In addition, depending on the species, the maintenance of a captive population can be difficult or impossible to attain, constraining the design of experiments and laboratory tests.

The use of sentinel avian species to detect potential threats to human health is not recent. For example, canaries were used in coal mines for centuries to detect coal damp before this gas overcame coal miners (Burrell and Seibert, 1916; Schwabe, 1984). Birds drew great attention in the 1960s and 1970s as sentinel species for organochlorine pesticides, particularly DDT, when it was discovered that exposure to these pesticides resulted in eggshell thinning (Hickey and Anderson, 1968; Ratcliffe, 1970). Since then, a wide variety of avian species has been used as sentinels. These include raptors such as bald eagles (*Haliaeetus leucocephalus*), peregrine falcons (*Falco peregrinus*), American kestrels (*Falco sparverius*), and sparrow hawks (*Accipiter nisus*), and piscivorous species such as brown pelicans (*Pelecanus occidentalis*), great blue herons (*Ardea herodias*), cormorants, gulls, and terns, all useful due to their high position in the food web (Grasman *et al*., 1998). Owl species have also been suggested as good candidates for sentinels (Gervais and Anthony, 2003). Species such as the bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix coturnix japonica*), Eastern bluebird (*Sialia sialis*), European starling (*Sturnus vulgaris*), tree swallow (*Tachycineta bicolor*), and various warblers that use natural or man-made cavities for nesting also can be useful sentinels (Mayne *et al*., 2004; McCarty, 2002; Romijn *et al*., 1995). A number of avian species are commercially available as either eggs or adults, including bobwhite quails (*Colinus virginianus)*, mallard ducks (*Anas platyrhynchos*), and ring-necked pheasants (*Phasianus colchicus*), allowing researchers to perform experimental exposure of adults to contaminants for single (see references in Table 1) or multigenerational studies (Heinz, 1979) and conduct egg-injection and incubation studies to assess developmental effects of environmental contaminants (Ottinger *et al*., 2001; Quinn, 2008). A number of these studies have focused on endocrine disruption. Indeed, a recent, specific use for wildlife sentinels is to detect contaminant exposure affecting the endocrine system, and this use deserves research emphasis and priority funding (DeRosa *et al*., 1998). However, there is a marked bias of this research in addressing the estrogenic or antiandrogenic properties of pollutants and their subsequent effects on gender phenotype and on reproductive capability. As a consequence, there has been very little attention to other endocrine systems (see below), despite evidence indicates that adrenal function (as well as, e.g., thyroid function) may be adversely affected by chemicals in the environment (Table 1).

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Table 1 A Survey of Studies Reporting Adrenocortical Measurements in Birds Exposed to Environmental Contaminants

Chemical class	Chemical/s	Exposure	Species	Age status	Parameter		Stressor	Variables	References
					B	SI			
Petroleum hydrocarbons	Crude oil	E	Mallard (*Anas platyrhynchos*)	J	↓			Sex	(Rattner and Eastin, 1981)
	Crude oil	E	Mallard (*Anas platyrhynchos*)	A	○				(Rattner, 1981)
	Crude oil	E	Mallard (*Anas platyrhynchos*)	A	↓				(Harvey *et al.*, 1981)
	Crude oil	E	Mallard (*Anas platyrhynchos*)	J	↓				(Gorsline and Holmes, 1981)
	Crude oil (DF)	E	Herring gull (*Larus argentatus*)	C	↑				(Peakall *et al.*, 1981)
	Crude oil (DF)	E	Black guillemot (*Cepphus grylle*)	C	↑			(Peakall *et al.*, 1981)	
	Crude oil (DF)	E	Leach's storm-petrel (*Oceanodroma leucorhoa*)	A	○				
	Crude oil	E	Mallard (*Anas platyrhynchos*)	J	↓				(Gorsline and Holmes, 1982)
	Crude oil (DF)	E	Mallard (*Anas platyrhynchos*)	J	↓				(Gorsline and Holmes, 1982)
	Crude oil	E	Mallard (*Anas platyrhynchos*)	J	↓ [age]			Age	(Gorsline and Holmes, 1982)
	Crude oil	E	Mallard (*Anas platyrhynchos*)	U	↓				(Gorsline, 1984)
	Crude oil	F	Magellanic penguin (*Spheniscus magellanicus*)	A	↑ [sex]			Sex	(Fowler *et al.*, 1995)

Organochlorine compounds	*o,p′*-DDD	E	Chicken (*Gallus domesticus*)	C	↓						(Newcomer, 1959)
	DDTs	E	Chicken (*Gallus domesticus*)	J	↓						(Srebocan *et al.*, 1971)
	DDE	E	Chicken (*Gallus domesticus*)	J		↓	ACTH				(Gross, 1990)
	PCBs	E	Mallard (*Anas platyrhynchos*)	A	○						(Fowles and Trust, 1997)
	PCBs, PCDDs, PCDFs, CHCs	F	Herring gull (*Larus argentatus*)	E	↓ [PCBs, PCDDs, PCDFs]						(Lorenzen *et al.*, 1999)
	PCBs, DDE	F	Bald eagle (*Haliaeetus leucocephalus*)	C	○	↑ [location]	ACTH	Age, Sex, Location			(Bowerman *et al.*, 2002)
	PCBs	E	American kestrel (*Falco sparverius*)	A	↓	↓	HR	Age, Body condition			(Love *et al.*, 2003b)
	PCBs, PCDDs, PCDFs, DDE	F	Tree swallow (*Tachycineta bicolor*)	C	↓ [PCDFs]	○	HR	Body weight, Body lipids, Time of day, Year			(Martinovic *et al.*, 2003)
	PCBs	F	Tree swallow (*Tachycineta bicolor*)	A		○	HR				(Franceschini *et al.*, 2005)
	PCBs	F	Tree swallow (*Tachycineta bicolor*)	C		↑, ↓ [year]	HR, ACTH	Year			
	p,p′-DDT	E	Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelli*)	A	○	○	FA, FL				(Scollon *et al.*, 2004)
	p,p′-DDE	F	Tree swallow (*Tachycineta bicolor*)	C	○	○, ↑ [year]	HR, ACTH	Body weight, Sex, Daily temperature, Collection date, Year			(Mayne *et al.*, 2004)
	p,p′-DDE	F	Eastern bluebird (*Sialia sialis*)	C	↑	↓	ACTH	Body weight, Sex, Daily temperature, Collection date			

(*continued*)

Table 1 A Survey of Studies Reporting Adrenocortical Measurements in Birds Exposed to Environmental Contaminants (*continued*)

Chemical class	Chemical/s	Exposure	Species	Age status	Parameter		Stressor	Variables	References
					B	SI			
Organophosphorus compounds	Parathion	E	Bobwhite quail (*Colinus virginianus*)	A	○	↑	C		(Rattner *et al.*, 1982a)
	Parathion	E	Bobwhite quail (*Colinus virginianus*)	A	○			Time of day	(Rattner *et al.*, 1982b)
	Fenthion	E	Black duck (*Anas rubripes*)	A	○	↓	SW		(Rattner *et al.*, 1983)
	Methyl parathion	E	American kestrel (*Falco sparverius*)	A	↑	○	C		(Rattner and Franson, 1983)
	Temephos	E	Mallard (*Anas platyrhynchos*)	C	↑	○	C		(Fleming *et al.*, 1985)
	Triorthotolyl phosphate	E	Chicken (*Gallus domesticus*)	A	↑				(Foil *et al.*, 1985)
	Mixture of nonpersistent pesticides	F	Tree swallow (*Tachycineta bicolor*)	C	○	○, ↑ [year]	HR, ACTH	Body weight, Sex, Daily temperature, Collection date, Year	(Mayne *et al.*, 2004)
	mixture of non-persistent pesticides	F	Eastern bluebird (*Sialia sialis*)	C	↑	↓	ACTH	Body weight, Sex, Daily temperature, Collection date	
Metals	Cd	E	Mallard (*Anas platyrhynchos*)	J	[↑]				(Di Giulio and Scanlon, 1984)
	Cd	E	Mallard (*Anas platyrhynchos*)	J		[↑]	FA		(Di Giulio and Scanlon, 1985)
	Al	E	Chicken (*Gallus domesticus*)	C	○			Food	(Capdevielle *et al.*, 1996)
	Hg	F	Bald eagle (*Haliaeetus leucocephalus*)	C	○	○	ACTH	Age, Sex, Location	(Bowerman *et al.*, 2002)

Cd, Hg, Se	F	Common eider (*Somateria mollissima borealis*)	A			↑ [Cd], ↓ [Se] [year, sex]	HR	Sex, Year, Handling time	(Wayland *et al.*, 2002)
Cd, Hg, Se	F	Common eider (*Somateria mollissima borealis*)	A			[↓] [Se] [sex]	HR	Body weight, Sex, Handling time	(Wayland and Smits, 2003)
Hg	F	Tree swallow (*Tachycineta bicolor*)	C	○	○		HR	Body weight, Body lipids, Time of day, Year	(Martinovic *et al.*, 2003)
Cu, Pb*	F	Great tit (*Parus major*)	A			○		Body weight, Handling time, Food	(Eeva *et al.*, 2003)
Cu, Pb[a]	F	Great tit (*Parus major*)	C			○		Body weight, Handling time, Food	
Pb	E	Zebra finch (*Taeniopygia guttata*)	A	○				Sex, Ca	(Snoeijs *et al.*, 2005)
Hg	F	White ibis (*Eudocimus albus*)	A			○		Sex, Reproductive stage	(Heath and Frederick, 2005)
Cu, Pb, Zn, Ni, As[a]	F	Pied flycatcher (*Ficedula hypoleuca*)	A			○		Handling time	(Eeva *et al.*, 2005)
Cu, Pb, Zn, Ni, As[a]	F	Pied flycatcher (*Ficedula hypoleuca*)	C			○		Handling time	
Cu, Pb, Zn, Cd, As	F	White stork (*Ciconia ciconia*)	C	○	○	↑ [Pb] [brood size]	HR	Age, Body condition, Sex, Brood size, Location	(Baos *et al.*, 2006)

Indicated is the chemical class, chemical/s per se, the type of exposure (E, vs. F), the species, age status (A, J, C, E, U), the adrenocortical parameter/s measured (B, SI,), the type of experimental stressor, when applicable (HR, ACTH, C, SW, FA, FL,), and the variables (factors and/or covariates) controlled for in statistical analysis, if any (e.g., age, sex, body condition, etc.). Significant ($P < 0.05$) associations with baseline, stress-induced, or both corticosterone measures are indicated as follows: ↑, increase cort; ↓, decrease cort; ○, no effect; symbols within brackets indicate marginally significant effect ($0.05 < P < 0.1$) as indicated by authors (e.g., [↑] marginally significant increase in cort levels). If results are constraint to a particular chemical (when more than a single chemical is considered in the same study) or variable involved is also specified between brackets. When the study includes more than one species and/or age status, and/or chemical class, separate rows are used for each species, status, and chemical class. Full references are given in the literature section at the end of the chapter.

We assume that cort levels measured in blood samples taken after 2 to 3 minutes after capture indicate stress-induced responses (Romero and Reed, 2005). If no information is reported regarding the time elapsed since capture, we assume that blood samples were collected immediately after capture and, therefore, cort concentrations represent baseline levels.

[a]Metals reported in the area at higher concentrations.

Abbreviation: DF, distillation fractions tested; E, experimental; F, field; A, adult; J, juvenile; C, chick or nestling; E, embryo; U, unknown; B, baseline corticosterone levels; SI, stress-induced corticosterone levels; HR, handling and restraint; ACTH, adrenocorticotropic hormone; C, Cold; SW, Salt Water; FA, fasting; FL, flight.

ENDOCRINE TOXICOLOGY AND ADRENOCORTICAL STRESS RESPONSE

Endocrine toxicology is generally referred to the action of chemicals on the structure and function of a particular gland (commonly known as the target organ approach). However, the endocrine system, more than any other, regulates homeostatic balance and as a whole is sensitive to changes in the function of its constituent glands and nonendocrine organs such as the liver. Therefore, chemically induced changes in nonendocrine organs can affect the endocrine system, and thus compounds inherently toxic to the liver, kidney, or brain may also impair the normal functioning of the endocrine system (indirect toxicity). Furthermore, toxicological studies often focus on the damage induced by a chemical to an organ or tissue, leading to total or subtotal failure in function. In endocrine toxicology, as occurs in immunotoxicology, chemically induced increases in function can be as harmful as it is the loss or the decrease in function (see below). Provided these particularities, among the endocrine glands, the adrenals, and especially the cortex, is one of the organs most commonly affected by toxic substances (Ribelin, 1984). Without precluding direct toxicity, a reason why the adrenals are so commonly implicated in endocrine toxicological responses concerns their unique position in the regulation of the stress response. Endocrinologists have long been aware of the adrenal stress response and the functions that it serves in conditions of adversity (Selye, 1936).

What Is a "Healthy" Response to Stress?

Animals have evolved physiological mechanisms to adjust their life cycle to a changing environment. Among birds, for example, life-history stages such as development, dispersal, reproduction, and migration normally follow a cyclic pattern in concert with predictable environmental changes (e.g., seasons, day-night, tides), and the endocrine system plays a fundamental role in adjusting behavior, morphology, and physiology to maximize life-time individual fitness (Jacobs and Wingfield, 2000). But in addition to cyclic, predictable changes in environmental conditions, all habitats suffer nonpredictable perturbations that challenge individuals' homeostasis. Sudden weather inclemency, floods, droughts, decreased feeding resources, and outbreaks of parasites or predators, among others, can strongly modify environmental conditions and jeopardize individuals' development, reproduction, and survival. In order to maximize fitness, birds have also evolved endocrine mechanisms to cope with such noxious and energy demanding unpredictable situations (Romero, 2004; Wingfield, 2003). Activation of the hypothalamus-pituitary-adrenal (HPA) axis constitutes a well-preserved emergency response in vertebrates, and it orchestrates physiological and behavioral changes adequate to cope with nonpredictable changes in environmental conditions. Following exposure to a perturbation, the hypothalamus releases corticotropin-releasing hormone (CRH) and some other hormones (Fig. 1), which stimulate the pituitary to secrete adrenocorticotropic

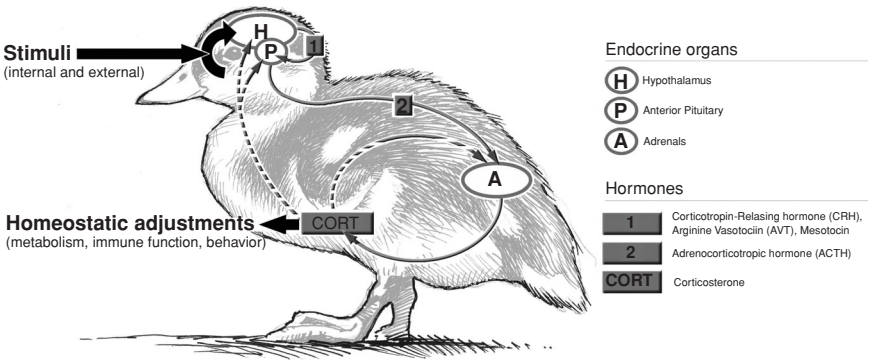

Figure 1 Schematic representation of the adrenocortical response to stress in birds. Following exposure to perturbations of exogenous or endogenous origin (e.g., predation attempts, energy imbalances; see upper black arrows), the hypothalamus (H) releases a number of hormones (Sheffield *et al*., 1998) including corticotropin-releasing hormone CRH. These in turn stimulate the anterior pituitary (P) to secrete adrenocorticotropic hormone ACTH (Fox, 2001) into circulation. In birds, the adrenals (A) respond to increased ACTH levels secreting corticosterone (CORT). Within minutes to hours following exposure to stress, the resulting corticosterone elevations promote multiple changes in physiology and behavior (lower black arrow) including increased gluconeogenesis, suppression of reproductive behaviors, regulation of immune function, irruptive migration, and increased night restfulness. These adjustments promote the maintenance of homeostasis through change (i.e., allostasis). Corticosterone secretion is subjected to negative feedback mechanisms, as indicated with dashed arrows. In addition to stress-related fluctuations, baseline corticosterone levels show circadian and circannual rhythms in birds, allowing endogenous regulation of numerous physiological processes.

hormone (ACTH) into circulation (Sapolsky *et al*., 2000). In birds, the adrenals respond to increased ACTH levels, secreting corticosterone. Within minutes to hours following exposure to stress, the resulting corticosterone elevations promote multiple changes in physiology and behavior including increased gluconeogenesis, suppression of reproductive behaviors, regulation of immune function, irruptive behavior, and increased night restfulness (Sapolsky *et al*., 2000; Wingfield and Ramenofsky, 1999; Wingfield and Romero, 2001). Increased corticosterone, therefore, constitutes a "healthy" or adaptive response to stress, as it promotes the maintenance of homeostasis through change [i.e., "allostasis" (McEwen and Wingfield, 2003)], priming physiological and behavioral adjustments aimed at maximizing immediate survival while suppressing nonessential activities. For this reason, experimental exposure to a number of physical noxious stimuli (such as capture and restraint, exposure to cold or heat, (Ramade and Baylé, 1980; Freeman and Manning, 1984; Blas *et al*., 2005) is a frequently used protocol to assess adrenocortical function in birds (Fig. 2). Elevated corticosterone levels following **F2**
short-term exposure to stress is therefore the expected response among healthy

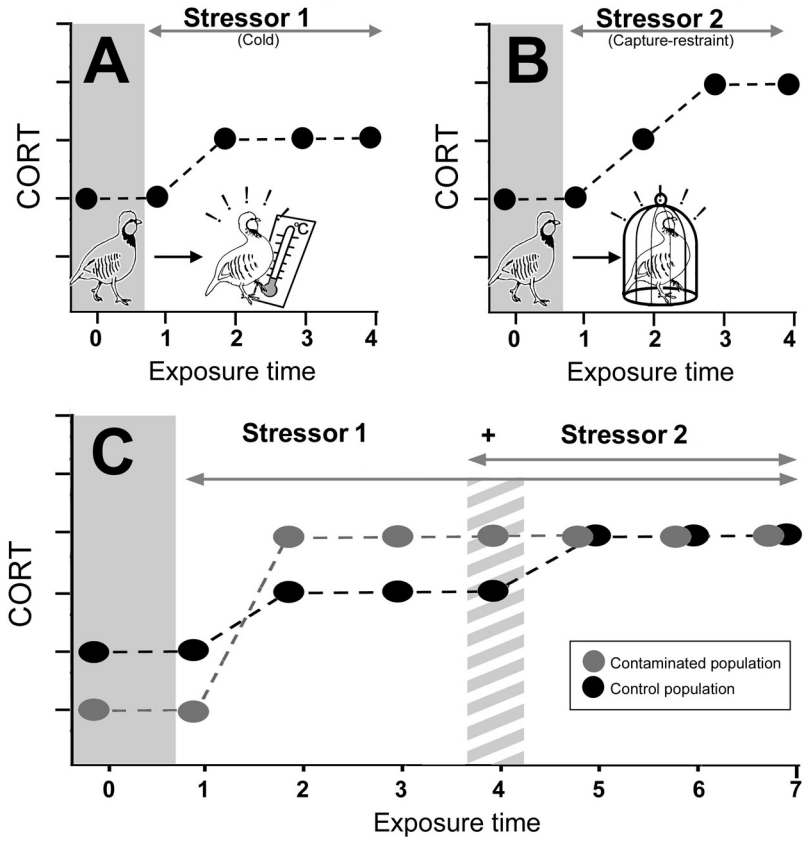

Figure 2 Assessing the response to stress: The importance of adequate baseline levels estimation. Exposure to a wide array of noxious stimuli activates the HPA axis of birds, which triggers a rapid elevation of the circulating levels of corticosterone. A widely used protocol aimed at assessing this response in birds consists on inducing experimental stress by means of exposure to a standardized perturbation, such as cold –(A), or capture and restraint –(B). This protocol is accompanied by the collection of several blood samples at predetermined intervals of time (*X*-axes). Subsequent determination of the concentration of corticosterone (*Y*-axes) in the collected samples allows an objective assessment of the individuals' time-course patterns of response, providing an objective physiological record to establish comparisons among populations and to study associations with behavioral and toxicological data. The collection of an initial blood sample shortly following stress induction is required to assess baseline corticosterone titers (i.e., resting levels, gray area). Ideally, this sample should be collected before exposure to experimental stress, but because corticosterone elevations do not occur immediately, blood samples collected within the first 2 to 3 minutes provide a valid estimation of baseline titers. After this brief time lag, corticosterone levels rapidly elevate (i.e., stress-induced or acute levels) over the course of 30 to 60 minutes. The magnitude of the response depends upon the type of stressor (e.g., **A** and **B**; cold vs. capture-restraint); and experimental protocols sometimes

individuals. It is less clear, however, whether chronic corticosterone elevations in response to prolonged or repeated exposure to stressful situations constitutes an adaptive response. Under specific scenarios (e.g., a fish in a contaminated pond, a wild bird caged, exposed to intense parasitism or to severe food shortages, a mammal exposed to social subordination), the ability of an individual to avoid a perturbation may not be possible despite activation of emergency responses. Animals may then habituate to the perturbation and decrease activation of the HPA axis [e.g., habituation to capture and handling, (Love *et al*., 2003a), or to captivity (Cabezas *et al*., 2006)], but depending on the quality of the stressful stimuli, this may not be possible (e.g., exposure to chronic malnutrition). Chronic (maintained from days to weeks) elevation of corticosterone levels may then promote catabolism up to the point of depleting fat stores and waste structural protein mass (e.g., muscle), and also inhibit the reproductive system, suppress growth and the immune system, disrupt second cell messengers, and provoke neuronal cell death (Sapolsky *et al*., 2000; Sapolsky, 1992; Wingfield and Romero, 2001). Numerous studies provide evidence for these and other deleterious effects of long-term experimentally elevated corticosterone (Joseph and Ramachandran, 1993; Kitaysky *et al*., 2003; Martin *et al*., 2005). However, it should be noted that this sort of experimental manipulation does not occur in a context of chronic stress; individuals subjected for a long-term to corticosterone elevations, normally show decreased fitness compared to controls (sham-implanted), provided that none of the experimental groups are exposed to a chronic perturbation. In other words, chronic corticosterone elevations may be maladaptive when there is no reason to activate emergency responses, but if the individuals are chronically exposed to a severe perturbation, this response could still be the best to a bad situation.

Figure 2 (*continued*) comprise different stressors sequentially applied to the same individuals (**C**). Despite acute corticosterone levels may be further elevated following exposure to a second source of stress (i.e., after time 4 in **C**), blood samples collected shortly after this time (dashed gray area) should not be considered true baseline levels. Such consideration would otherwise lead to an incorrect interpretation of the results, as illustrated in **C**; individuals from the population exposed to contaminants (gray dots) would be described as having higher baseline levels and being unable to respond to stress, when in fact they had a lower baseline and a faster corticosterone elevation compared to control birds (black dots). This observation is also relevant when comparing the stress response among populations, because uncontrolled local perturbations (e.g., inclement weather, parasites) may generate corticosterone elevations prior to the experimental exposure to stress (i.e., before the dashed gray area in **C**) potentially misleading the interpretation of contaminant-related effects. For all these reasons, it is always advisable to collect information on contaminant exposure on an individual basis (rather than just using a population mean), work concurrently on the different study populations (to avoid seasonal and interyear variability), and avoid intense sampling over a short period of time (to dilute the effects of episodic stress such as predation attempts in colonial birds, or weather-related variability).

Chemical Stressors or Endocrine Disruptors

Field endocrinologists often assess circulating glucocorticosteroid levels (or related adrenocortical parameters, such as fecal corticosteroid metabolites) in wild vertebrates and use this endocrine parameter as a biomarker of exposure to environmental stress (Walker *et al*., 2005). Despite increased baseline corticosterone levels may ultimately reflect activation of an emergency response, the adrenocortical system is not stressor-specific. Elevated baseline glucocorticosteroid levels are expected to occur among individuals or populations exposed to decreased food resources (Kitaysky *et al*., 1999; Kitaysky, 2001), reduced habitat quality (Marra and Holberton, 1998; Suorsa *et al*., 2003; Wasser *et al*., 1997), and increased anthropogenic pressure (Mullner *et al*., 2004; Walker *et al*., 2005; Walker *et al*., 2005), among others. Ultimately, all these perturbations share a common property; they increase the energy demands of the individual and, therefore, a corticosterone response helps to maintain homeostasis through promoting changes in physiology and behavior (McEwen and Wingfield, 2003). But what happens when the individual or population is exposed to environmental contaminants? Our literature survey shows that an array of chemicals also elicit corticosterone elevations in birds (Table 1); is this enough to label a chemical as "endocrine disruptor" or should we just consider it to be one more "stressor"? Let us compare these terms.

Stressors, Perturbations, Modifying Factors, and Some Other Names

The terminology involved in the biomedical literature studying stress can be confusing, because the same term "stress" has been traditionally used to describe the noxious stimuli that an individual is exposed to, the physiological and behavioral coping responses, and the overstimulation of the coping responses that result in disease (Romero, 2004). But even constraining our discussion to definitions of the term "stressor" related the first meaning, i.e., "stressor is a noxious or unpredictable stimuli that causes a stress response" (Romero, 2004), different authors use a very varied terminology to define and classify stressors.

Romero (Romero, 2004) differentiates between "acute stressors" (those that last a short period of time, such as predator attacks, dominance interactions, and storms) and "chronic stressors" (i.e., the latter ones when they persist on time, e.g., long-term subordinance, famine).

Wingfield (Wingfield, 2003) refers to stressors using the terms "modifying factors" or "labile perturbation factors" that are defined as unpredictable events in the environment able to trigger a facultative emergency life-history stage, which redirects the individual away from the normal life-history stage (e.g., winter, breeding, moult) into a survival mode. The life-history emergency state is characterized by increased secretion of glucocorticosteroids, and some examples of these unpredictable events include severe storms, predator pressure, and human disturbance. These environmental perturbations can last long-term (e.g., human disturbance, global climate change) or be transient ("labile"). Within transient or labile perturbations, Wingfield discriminates two groups: Indirect (rapid events

that do not reduce food or access to it, e.g., nest predation, a sudden hail storm) and direct (longer-term that force the individual to interrupt a previous life-history stage, for example, reduced food supply, drop in social status, disease, predator influx), and includes pollution/endocrine disruptors into the latter category of direct labile perturbations.

McEwen and Wingfield (McEwen and Wingfield, 2003) present three new concepts that have generated some controversy (Dallman, 2003; Walsberg, 2003): allostasis (the maintenance of homeostasis through change), "allostatic load" (the measure of how hard an individual must work to accomplish a normal life-history task, such as the energy requirements for breeding), and "allostatic overload" (the state in which energy requirements exceed the capacity of an individual to replace that energy from the environment), and suggest to use the term "stress" only referred to stimuli that require an emergency energetic response [i.e., the equivalent to stressor according to Romero, 2004 (Romero, 2004)].

Pottinger (Pottinger, 2003) defines stressor as a "destabilizing stimulus of external or internal origin," and classifies stressors as physical (abiotic, such as temperature, wind, habitat alteration, etc., or biotic, such as conflict, predator, or parasite damage), chemical (e.g., contaminants), physiological (starvation, disease, dehydration), and psychological (threat of predation, intra- and interspecies conflict, territoriality).

Endocrine Disruptors

Originally, the concern over endocrine disruption was based almost entirely on perceived effects of chemicals on the reproductive system and it was usual to refer to these chemicals as estrogen mimics or estrogenic substances. Later, chemicals were found that could block estrogenic responses (antiestrogenic) or androgenic responses (antiandrogens), and it was soon recognized that some substances could affect other elements of the endocrine system via interaction with hormones other than sex steroids (WHO/IPCS., 0000).

The term endocrine disruptor is now preferred because it allows inclusion of health effects thought to result from interference with any part of the endocrine system. Although there are several different definitions for this term in current use (Phillips and Harrison, 1999), the final report of the U.S. EPA's Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC, 1998) defines an endocrine disruptor as "an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle."

A major difficulty that has been encountered with this definition (identified as a particular problem by EDSTAC) is the definition of the term "adverse." For a chemical to be judged as an endocrine disruptor, it is important to show that the recorded response has an adverse effect on the health or reproductive capability of affected organisms or populations and that this response does not fall within the normal range of physiological variation. This premise may help us to answer the

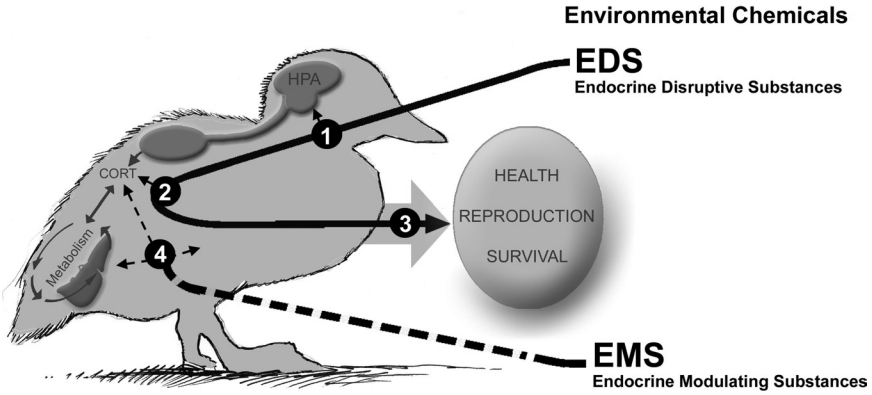

Figure 3 Environmental chemicals affecting the HPA axis. Although there is growing evidence that numerous human activities–derived chemicals present in the environment can affect the normal function of the stress axis, just a few of these substances can be categorized as endocrine disruptors following the current definition of this term (WHO/IPCS., 0000). Gray letters provide a schematic representation of the HPA system of a bird. Activation of this endocrine axis results in the secretion of corticosterone (CORT), which modulates numerous physiological functions such as metabolism (e.g., increasing hepatic glucose output through enhanced gluconeogenesis). Normal regulation of circulating corticosterone levels allows birds to maintain homeostasis, and therefore maximizes individual fitness, as represented with the broad gray arrow pointing to the main fitness traits (i.e., health, reproduction and survival). In order to classify a chemical as "endocrine disruptor" of the stress axis (EDS, see continuous black arrow) the following circumstances have to be proven: (1) the primary site of action is the endocrine system; (2) the structure or function(s) of the endocrine system is altered (e.g., corticosterone levels are abnormally increased or decreased); and (3) it causes adverse health effects at the level of the organism, its progeny, or the population. Demonstration of these three conditions requires an extensive knowledge of the specific mechanisms of action of each chemical, and this is extremely difficult to attain in field and environmental studies. In addition, the HPA-response is not stressor-specific and, therefore, can be indirectly activated if exposure to a given chemical jeopardizes homeostatic balance (e.g., exerting its action over other organs—such as the liver, or over other physiological processes—such as metabolism). As a consequence, despite many chemicals have been shown to elicit circulating corticosteroid levels above or below "normal" values, unless we relax the current definition of EDS they have to be considered as "chemical stressors" or "endocrine modulating substances" (EMS, see dashed black line).

question formulated above; as mentioned, the elevation of corticosterone levels following exposure to a chemical stressor could simply be the normal or expected stress response, and differ little from the response to capture or predation attempts. Thus, provided that such response does not exceed the homeostatic capacity of the individual and/or populations, the chemical might be just one more stressor, rather than an endocrine disruptor (Fig. 3) (EDSTAC, 1998).

A second problem associated to the definition of endocrine disruptor concerns the mechanism(s) of action of the chemical; disruptors should primarily affect the endocrine system, thus excluding from this classification those chemicals causing overt toxicity in other body systems and indirectly affecting endocrine function. With regards to the HPA axis, the multiple levels of control over the stress response imply many potential sites of action for chemicals affecting or disrupting adrenal homeostasis (Pottinger, 2003). For example, chemicals may influence the negative feedback control loops of the adrenals through modifying hepatic metabolism, causing changes in glucocorticoid secretion (Rehulka and Kraus, 1987). Because such alterations in adrenal function constitute indirect responses (i.e., the primary site of action is not the endocrine system), these chemicals should not be called endocrine disruptors. In contrast, changes in ACTH secretion that result from chemical actions on the brain or pituitary gland and that will also alter secretion of glucocorticoids (Hadley *et al*., 1990; Spindel *et al*., 1983), constitute a secondary response to chemical actions at extra-adrenal endocrine sites and, therefore, might be considered endocrine disruption (provided that they cause adverse health effects on individuals and/or populations). At this point, it is important to note that the underlying mechanism(s) of action of most chemicals affecting the adrenocortical response to stress is still poorly understood, especially when the evidence of effects comes from field studies (Baos *et al*., 2006; Wayland *et al*., 2002).

On the other hand, although transient elevations in circulating corticosterone concentrations are highly adaptive through facilitating short-term responses to stressors (by promoting behavioral changes or mobilizing energy reserves), chronic corticosterone elevations can have very relevant deleterious consequences such as fat-stores depletion, muscle waste, inhibition and suppression of growth, reproductive and immune systems, disruption of second cell messengers, induction of neuronal cell death (Sapolsky *et al*., 2000; Sapolsky, 1992; Wingfield and Romero, 2001). Similarly, lower circulating levels of corticosterone can result in an inability to respond to stress, reduced gluconeogenesis, and stimulation of the immune system (Colby and Longhurst, 1996) that may ultimately affect fitness. Thus, abnormal responses in both directions, i.e., increasing or decreasing corticosterone levels as consequence of primary or secondary toxicity of chemicals acting directly or indirectly on the endocrine system might be equally harmful for individuals and/or populations. The lack of basic knowledge on the feedback loops and the boundaries of an organism's homeostatic range, affects our ability to place in context the significance of a particular observation obtained in the field or even in the laboratory. Clearly, the boundaries of endocrine disruption, and particularly those concerning adrenocortical function cannot be clearly delineated.

For the purpose of our review, we will focus on chemicals in the environment that can increase or decrease adrenocortical function (i.e., plasma baseline and/or stress-induced corticosterone levels), using the term endocrine-modulating substance (EMS) to include the terms "chemical stressors," "endocrine active substances," and "endocrine disruptors" (Fig. 3) (EDSTAC, 1998). This is a relatively

broad consideration of the chemicals of concern and reflects the difficulty to face research into adrenal "disruptors," especially when working with wildlife.

ENDOCRINE MODULATING CONTAMINANTS IN THE ENVIRONMENT AND THE ADRENOCORTICAL RESPONSE TO STRESS

In this section, we will review the literature on adrenocortical stress response in birds exposed to environmental contaminants, with the goal of finding common patterns of responses that may allow us to reach conclusions and make inferences, as well as to identify research gaps and delineate future research directions.

A survey of the published studies reporting adrenocortical measurements [i.e., plasma baseline (or basal) and stress-induced (or acute) corticosterone concentrations] in avian species exposed to chemicals reveals that more than 90% of them (N = Rattner *et al*., 1982a) deal with exposure to petroleum hydrocarbons, organochlorines (PCBs, PCDDs, PCDFs, and persistent organochlorine insecticides, such as DDT and its metabolites), metals, and organophosphorous compounds (Table 1).

Petroleum Hydrocarbons

The hydrocarbons considered in this review involve various compounds present in crude petroleum that are frequently released into the environment following major oil spills or slow seepage from natural deposits, oil industry storage, and extraction sites. Acute, subacute, and chronic exposure of birds may occur in nature through the oiling of plumage and through the ingestion of oil via feeding or preening.

The impact of petroleum hydrocarbons on avian adrenocortical function received considerable attention during the 1980s (Table 1). Most of the studies reporting plasma corticosterone in birds exposed to crude oil have been conducted experimentally on mallard ducks, and declines in circulating corticosterone concentrations have characterized their responses to ingested petroleum-contaminated food (see references cited in Table 1). Studies in vivo and in vitro have confirmed that the petroleum-induced decreases in plasma corticosterone concentration reflect diminished adrenocortical activities, due primarily to a suppression of the corticotropic responsiveness of cells in the inner zone of the adrenal gland (Gorsline and Holmes, 1982). However, it is also feasible that petroleum-induced changes in liver function may indirectly influence adrenocortical function in contaminated birds. As in many other organisms exposed to hydrocarbon pollutants, the liver of birds consuming petroleum-contaminated food develops an increased ability to metabolize the circulating contaminants (Gorsline, 1981; Miller *et al*., 1978). This is accomplished through the action of a substrate-inducible mixed function oxidase system. However, although the primary function of this system is to rid the organism of the contaminants, it may also accelerate the turnover of some endogenous substrates, such as steroid hormones (Peakall, 1967). Thus, the low plasma corticosterone concentrations found

in birds exposed to petroleum-contaminated food may have been caused by two distinct types of effects— one involving a diminished responsiveness to ACTH in the adrenals, and the other comprising an enhanced metabolism of circulating hormones (Gorsline and Holmes, 1981).

Although different crude oils seem to produce qualitatively similar effects on plasma corticosterone concentrations, the magnitude of the responses evoked in laboratory-maintained ducks varied considerably (—Rattner and Eastin, 1981; Harvey *et al*., 1981; Gorsline and Holmes, 1981). Many factors may have been responsible for this variability. For example, the chemical composition of the crude oil and the environmental conditions under which the birds were maintained, could both contribute to the reported quantitative differences in response. Also, the magnitude of the perceived response may vary with the time of the day when blood samples were taken and hormone concentrations were compared (Gorsline and Holmes, 1981).

Unlike many other pollutants, crude oils are complex mixtures of different types of hydrocarbons, and thus it cannot be assumed that their toxicities are always attributable to a particular class of compounds. Gorsline and Holmes (Gorsline and Holmes, 1982) found differences in plasma corticosterone concentrations among different distillation fractions of crude oils, but were unable to attribute this effect to specific hydrocarbon compounds present in any of the fractions.

The lower molecular weight constituents of crude oil, particularly the aromatic hydrocarbons, have often been assumed to be responsible for most of the adverse effects seen in contaminated organisms (Gorsline and Holmes, 1982). In growing mallard ducks, decreases in plasma concentration of corticosterone after chronic ingestion of crude oil were most apparent in birds fed the oil with apparently greater aromatic content and was somewhat dose-dependent (Rattner and Eastin, 1981).

It is of particular interest that the extent of the reported decreases in adrenocortical function, even in response to the ingestion of a particular crude oil, may also differ among birds exposed to contaminated food for only a few days and those that have consumed the same food for several months (Gorsline and Holmes, 1981; Rattner and Eastin, 1981). In these instances, it is impossible to determine the exact reason for the differences in evoked change, as they may be due primarily to the duration of the exposure or reflect modifications in response due to aging (Gorsline and Holmes, 1982). Gorsline and Holmes (Gorsline and Holmes, 1982) reported larger decreases of plasma corticosterone in younger exposed ducks than in older birds, while no changes in plasma corticosterone concentration occurred with aging in the control birds. Thus, age seems to be an important factor determining the degree of the hypoadrenocorticalism developed following exposure to petroleum-contaminated food. Rattner (Rattner, 1981) showed no effects on corticosterone levels in adult mallards exposed to petroleum contaminated food for 7 days, suggesting that adults can tolerate oil-contaminated food better than hatchling and young growing birds. Similarly (Peakall *et al*., 1981), failed to find effects on plasma corticosterone levels in adult leach's petrels (*Oceanodroma leucorhoa*)

dosed with weathered oil in a semifield experiment where birds were recaptured, while nestlings herring gulls (*Larus argentatus*) and black guillemots (*Cepphus grille*) sampled in the same study showed significant endocrine effects. However, contrary to previous experimental reports, oil-dosed nestlings of both species of seabirds showed higher levels of corticosterone compared to control birds. Fowler et al. (Fowler *et al*., 1995) also found elevations of corticosterone levels in lightly oiled female Magellanic penguins (*Spheniscus magellanicus*) at the beginning of the breeding season following an accidental crude-oil spill. The authors argued that oiled penguins have to face heavy energetic demands and that elevated corticosterone levels are consistent with the role of this hormone in mobilizing energy substrates. Coincidentally, the latter two studies were the only ones that were conducted under field conditions, and both reported an elevation of corticosterone levels after exposure to petroleum hydrocarbons. In natural settings, birds must face considerable fluctuations in environmental conditions such as changes in food availability. The exposure to other stressors may interact with petroleum effects on the adrenocortical stress response, resulting in higher levels of several hormones. In this regard, Peakall et al. (Peakall *et al*., 1981) reported increases in both corticosterone and ACTH levels in oil-dosed nestling gulls. Adrenocorticotropic hormone is released by the pituitary in response to low glucocorticoid levels (Fig. 1). Because elevated glucocorticoids inhibit ACTH release (through negative feedback), in the absence of severe pathology, only "stress" will result in both elevated plasma corticosterone and elevated ACTH. On the other hand, the composition of the crude oil may also contribute to explain different results between field and experimental studies. Nestling herring gulls showed increased corticosterone levels only after exposure to certain crude oil or aromatic fractions (Peakall *et al*., 1981).

Organochlorine Compounds

Under this term, we will refer to polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and organochlorine insecticides such as DDT and its metabolites. In general, these chemicals are characterized by being highly lipophilic and showing low solubility in water, which facilitates their accumulation in fatty tissues and fat stores, often at increasing concentrations in animals occupying the higher levels of the food web (Borga *et al*., 2001). All share a high environmental persistence.

Polychlorinated biphenyls are commercial mixtures of related compounds (congeners), which were once used (in many countries, the use of PCBs is now banned or severely restricted) as dielectric fluids, heat transformer fluids, lubricants, vacuum pump fluids, as plasticizers (e.g., in paints), and for making carbonless copy paper. Major sources of pollution are or have been manufacturing wastes and the careless disposal or dumping of the liquids referred above (Waid, 0000).

The best known member of PCDDs (there are Sapolsky, 1992 possible congeners of PCDDs) is 2,3,7,8-tetrachlorodibenzodioxin (Becker, 2003; Fox, 2001; Kushlan, 1993; Lower and Kendall, 1992-TCDD), usually referred to simply as "dioxin." This is a compound of extremely high toxicity to mammals. Polychlorinated dibenzofurans are similar to PCDDs both in structure and origin. Both PCDDs and PCDFs are not produced commercially, but are unwanted by-products generated during the synthesis of other compounds. Dioxins are also formed during the combustion of PCBs (fires or chemical waste disposal) and by the interaction of chlorophenols (used as wood preservatives) during disposal of industrial wastes (e.g., pulp mill effluents). Like PCBs, PCDD residues have been detected widely in the environment (especially in the aquatic environment), albeit at low concentrations, e.g., in fish and fish-eating birds.

Organochlorine insecticides such as DDT are highly persistent in their original form or as stable metabolites. DDT was used mainly for vector control of insects transmitting diseases during the Second World War, but came to be very widely used thereafter for the control of agricultural pests, vectors of diseases (e.g., malarial mosquitoes), ectoparasites of farm animals, and insects in domestic and industrial facilities. By 1990s, the use of these compounds for most purposes had been banned on the grounds of perceived human health risks or hazards to the environment. However, some of these compounds continue to be used in some developing and tropical countries, for example, to control vectors such as the malarial mosquito. The very marked persistence of compounds such as *p*,*p*′-DDE has ensured that significant residues are still present in once heavily contaminated soils and/or sediments and will only slowly disappear over the decades to come. These residues are still slowly released into aquatic and terrestrial food webs and can reach significant concentrations in animals at higher trophic levels.

Among the environmental contaminants, persistent organochlorine compounds have received great attention in relation to endocrine modulation or disruption in avian species (WHO/IPCS., 0000). However, most of the studies in this regard have dealt with their well-known estrogenic and/or antiandrogenic properties (Guillette, 2006; Vos *et al*., 2000); being comparatively scarce, the literature published on organochlorine stress-related endpoints. Moreover, except for a couple of experimental studies with poultry (DDTs) carried out in late 50s (Newcomer, 1959) and early 70s (Srebocan *et al*., 1971), most research on the adrenocortical stress response in birds exposed to organochlorine contaminants have been conducted during the last decade. Over this period, more than a half of the published scientific reports correspond to field studies (Table 1) where, in addition to basal corticosterone levels, the authors have usually incorporated estimates of stress-induced response. Circulating corticosterone concentrations in blood collected immediately after capture (i.e., basal corticosterone) were assumed to reflect environmental stress as opposed to the stress-induced response measured after a standardized capture, handling, and restraint protocol; exposure to a physical stressor designed to produce an increase in circulating corticosterone (e.g.,

cold); or ACTH injection (Fig. 2). Stress-induced corticosterone concentration, usually in conjunction with basal corticosterone is used as a correlate for a functional HPA axis (Norris, 2000; Hinson and Raven, 2006). Furthermore, challenge with an exogenous ACTH injection allows distinguishing adrenal gland response from nonadrenal causes of changes in circulating corticosterone concentrations, which might help to identify the mechanism of toxicity.

Despite a prolific use of DDT for more than a quarter century, most of the residual DDT in the environment exists as *p,p′*-DDE (in commercial DDT, 70–80% corresponds to *p,p′*-DDT). Other metabolites such as *o,p′*-DDE, *p,p′*-DDD, and *o,p′*-DDD are also present in lesser amounts. Exposure to technical grade DDT (75% *p,p′*-DDT and 25% *o,p′*-DDT), *p,p′*-DDT, and *o,p′*-DDD has been shown to decrease basal corticosterone in chickens (Newcomer, 1959; Srebocan *et al*., 1971), with dose-dependent reductions in individuals fed technical grade DDT for several weeks (Srebocan *et al*., 1971). Dose (or the environmental level of exposure) and duration of exposure are factors argued by some authors to be the cause of failing to detect significant effects of DDTs on corticosterone concentrations either in experimental (Scollon *et al*., 2004) or field studies [(Mayne *et al*., 2004; Lorenzen *et al*., 1999) in tree swallow]. Except for DDE, Lorenzen et al. (Lorenzen *et al*., 1999) found negative correlations between basal corticosterone in herring gull embryos from the Great Lakes and environmental levels of PCBs (total and nonortho PCBs), PCDDs, and PCDFs measured in yolk sacs. Similarly, Martinovic et al. (Martinovic *et al*., 2003) found negative correlations between basal corticosterone levels and PCDFs in nestling tree swallows sampled in two consecutive years, although they also reported no differences between exposed and reference sites in basal or stress-induced corticosterone concentrations after 10 minutes of handling and restraint. As mentioned earlier, the functionality of HPA axis through this procedure, or by the injection of ACTH, has been explored in several studies in which birds were exposed to organochlorine compounds, and their response compared with control or reference animals. In general, although both exposed and control (or reference) birds responded to the standardized handling and restraint procedure (or ACTH injection) by an increase in secretion of corticosterone (Love *et al*., 2003b), negative effects of PCBs and DDE on stress-induced response have been reported in both field and experimental studies (Gross, 1990; Love *et al*., 2003b; Mayne *et al*., 2004). Nevertheless, in some instances, associations are not straightforward. For example, Bowerman et al. (Bowerman *et al*., 2002) found that exposure to DDE and PCBs in nestling bald eagles was associated with lesser induction of plasma corticosterone on a regional level (i.e., Great Lakes or Interior breeding area) when challenged with ACTH. However, they also reported that increases in corticosterone induction were positively related to increases in either DDE or PCBs. In two consecutive years of study, Franceschini et al. (Franceschini *et al*., 2005) found that tree swallow nestlings chronically exposed to high PCB levels exhibited an increase in poststress corticosterone concentrations in comparison with birds from reference sites during the first year;

however, lower levels of corticosterone after ACTH injection were reported in the second year. In a study examining stress response in songbird nestlings coexposed to *p,p′*-DDE residues and a mixture of nonpersistent pesticides in apple orchards, Mayne et al. (Mayne *et al*., 2004) found interspecies differences in both basal and stressinduced response between tree swallows and Eastern bluebirds sampled in sprayed and reference sites (Table 1). Thus, while basal corticosterone levels in nestling tree swallows was not affected by the exposure to pesticides, and levels of corticosterone secretion post-ACTH stimulation were increased in the sprayed orchards, exposed bluebird nestlings had higher levels of basal corticosterone and were less responsiveness to challenge with ACTH than reference birds (Table 1). Furthermore, stress-induced corticosterone concentrations in bluebirds were negatively associated with *p,p′*-DDE levels in eggs. From these results, and since eastern bluebird eggs contained much higher concentrations of *p,p′*-DDE than tree swallow eggs, authors concluded that modulation of HPA axis in the tested songbird chicks was mostly associated with high persistent pesticides, i.e., *p,p′*-DDE. Results from laboratory studies have established that DDT metabolites are potent toxicants in the adrenal cortex of birds (Jönsson et al., 1994). The high lipid content of the cortical tissue of the avian adrenals has a high affinity for the metabolites of DDT; the primary metabolite in adrenal tissue of chickens dosed with DDT was *p,p′*-DDE (Srebocan *et al*., 1971). Disruption of cortical cell activity by *p,p′*-DDE ultimately inhibiting steroidogenesis has been shown previously in mammals (Lund, 1994). Alternatively, mixed-function oxidase activity may be induced by high levels of *p,p′*-DDE. This, potentially, could increase the metabolic clearance rate of corticosterone, activating feedback mechanisms and prolonging the release of ACTH from pituitary corticotropes. Chronic stimulation of cortical tissue would have the effect of exhausting adrenal cortical cells and dampening the response to ACTH injection.

Organophosphorous Compounds

Organophosphorous compounds are organic esters of phosphorus acids that act as nerve poisons (neurotoxins) due to their ability to inhibit the enzyme acetylcholinesterase. Today, a large number of organophosphorous compounds are marketed as insecticides, being extensively used for the control of agricultural pests and disease vectors. They are more polar and water soluble than the main types of organochlorine insecticides, although their water solubility is highly variable. Despite their lipophilic character, they are, in general, less stable than organochlorine insecticides and more readily broken down by chemical or biochemical agents. Thus, they tend to be relatively short-lived in the environment and in the tissues of homeothermic animals, being environmental hazards largely, but not exclusively, associated with short-term (acute) toxicity. It is remarkable that, despite their short-lives, some organophosphorous insecticides are highly toxic to birds and small mammals for brief periods after application, occasionally

affecting local wildlife populations (e.g., secondary poisoning in raptors, (Mineau *et al*., 1999).

Our literature survey reveals that a few number of studies have reported plasma corticosterone levels in birds exposed to organophosphorous compounds, and that almost all of them were published in a 4-year window (1982–1985), corresponding to experimental work conducted on adults of different avian species. The main aim of these investigations was to assess overt toxicity of organophosphorous compounds on different aspects of avian physiology, either alone or in combination with physical stressors such as cold (Rattner and Franson, 1983; Rattner *et al*., 1982a). Corticosterone was determined in most cases within routine plasma chemistries and, overall, results showed a dose-dependent increase in corticosterone concentrations after organophosphorous exposure (Table 1). Thus, Fleming et al. (Fleming *et al*., 1985) found increased levels of corticosterone only in the ducklings exposed to the highest (100 ppm) dietary temephos concentration. Similarly, subchronic ingestion of 100 ppm parathion for 10 days followed by exposure to mild cold (6 °C) for up to 48 hours resulted in two- to fivefold elevation of plasma corticosterone concentration in female bobwhite quails, yet birds receiving 0 and 25 ppm parathion were not affected (Rattner *et al*., 1982a). Acute exposure to methyl parathion also elevated plasma corticosterone concentration in adult American kestrels (Rattner and Franson, 1983). In the single and most recent study performed in the wild, Mayne et al. (Mayne *et al*., 2004) investigated the combined effects of several nonpersistent pesticides and the organochlorine insecticide *p*,*p*′-DDE on songbird nestlings' stress response. Tree swallow nestlings from pesticide-sprayed orchards showed higher levels of corticosterone after ACTH injection than chicks from reference sites; however, these results must be interpreted with caution since organophosphorous compounds were only one class of the chemicals included in the mixture of pesticides sprayed, and there was no correlation between stress-induced corticosterone concentrations and several estimates of pesticide exposure. Only Rattner et al. (Rattner *et al*., 1983) reported sustained corticosterone levels in ducks exposed to fenthion and receiving salt water for 12 days while nonexposed birds increased their levels of corticosterone. Thus, a reduced responsiveness of the HPA axis to organophosphorous exposure was suggested. In mammals, organophosphorous insecticides have been demonstrated to inhibit adrenal cholesterol esterification and hydrolysis, as well as hepatic steroid metabolism (Conney *et al*., 1971), and to reduce the rate of corticosteroidogenesis in vitro (Civen and Brown, 1974; Civen *et al*., 1977).

Metals

Metals are a large family of elements characterized by complex chemistry. Some metals are essential for normal physiological function, as integral parts of amino acids, nucleic acids, and structural compounds. Zinc, for example, is an essential component of at least 150 enzymes, Cu is essential for the normal function of

cytochrome oxidase, and Fe is part of hemoglobin. All essential metals have a "window of essentiality," within which dietary concentrations in animals have to be maintained if the organism is to grow and reproduce normally. The window of essentiality for some elements is very narrow (e.g., Se). Metals such as Cd, Hg, Pb, or As are referred to as nonessential because they do not have a known physiological function. In addition to being toxic above certain levels, nonessential metals such as Hg or Cd, may also affect organisms by inducing deficiencies of essential elements through competition at active sites in biologically important molecules. Such antagonism also occurs between essential elements.

The biological half-life varies for different metals. In mammals, the half-life of Cd is 20 to 30 years, while the half-life of As or Cr is a few hours or days. Their toxicity depends not only on dose and on length of exposure as occurs with other toxicants, but also on the ionic and chemical form (the species) of the metal and its bioavailability.

Metals are natural elements discharged into the environment by alteration of their geochemical cycles, through either human activities or natural processes such as volcanic eruptions or soil erosion. Mining and smelting activities, coal and petroleum combustion, and agricultural use of sludge from water treatment plants are important sources of contamination. The use of metal-based pesticides (e.g., lead arsenate) further contributes to environmental contamination. Acidification of watersheds by acid rain influences metal distribution in the ecosystem by promoting lixiviation of metals from soils into the aquatic compartment. Because their ecological half-life is long, and although their structure can be modified in the environment or in the animals by speciation and processes such as ionization, methylation, and binding to organic ligands, metals are classified as persistent contaminants. Moreover, their importance and widespread use in the manufacture of many products make contamination by metals ubiquitous.

Despite the extent to which biomarkers are able to provide unambiguous and ecologically relevant indicators of exposure to or effects of toxicants remains highly controversial (Forbes *et al*., 2006), over the past decade, the adrenocortical stress response has been widely considered within a suite of biomarkers chosen to reflect animal health and fitness in metal-exposed wild bird populations (Table 1). Our literature survey reveals that most of the studies performed in this regard have failed to detect significant effects of metal exposure on adrenocortical stress response (Table 1). However, it seems that the absence of significant results are more frequent for some elements, such as Hg (Bowerman *et al*., 2002; Heath and Frederick, 2005; Martinovic *et al*., 2003; Wayland and Smits, 2003; Wayland *et al*., 2002) than for others like Cd or Se (Di Giulio and Scanlon, 1984; Di Giulio and Scanlon, 1985; Wayland and Smits, 2003; Wayland *et al*., 2002). In any case, it is important to highlight that the number of publications per metal is very limited to reach definitive conclusions.

In a 3-year monitoring program of a breeding colony of common eiders (*Somateria mollissima*) in the Canadian Arctic, Wayland et al. (Wayland and Smits, 2003; Wayland *et al*., 2002) found negative relationships between hepatic

Se concentration and the stress-induced response in female eiders (Wayland and Smits, 2003; Wayland *et al*., 2002), while renal Cd concentration was reported to be positively related to plasma corticosterone levels in incubating fasted-females (Wayland *et al*., 2002). Although following the ecoepidemiological criteria (Fox, 1991), the lack of consistency in relationships among years was argued by the authors to avoid concluding that Cd exposure was related to the magnitude of the stress response in eiders, experimental studies conducted on mallard ducks provide support for a positive association (Di Giulio and Scanlon, 1985). Di Giulio and Scanlon (Di Giulio and Scanlon, 1985) showed that mallards simultaneously food-restricted and exposed to dietary Cd had higher (although marginally significant) concentrations of corticosterone compared to non-Cd exposed and non-food-restricted counterparts.

The functional tests are highly relevant to assess the situation in the wild where birds from contaminated sites must not only cope with the contaminant(s), but also must react appropriately to predators, conspecifics, and various environmental stressors, either chronic or acute, such as food deprivation, harsh weather conditions, etc. In a recent work conducted on white stork (*Ciconia ciconia*) nestlings exposed to metals subsequent to a mining accident in southwestern Spain, Baos et al. (Baos *et al*., 2006) showed no significant relationships between metals (Cu, Zn, Cd, Pb, As) and basal corticosterone concentration. However, maximum corticosterone concentration after a standardized handling and restraint protocol was positively related to low blood Pb levels, and singleton nestlings had higher levels of corticosterone than nestlings from multiple-chick broods. In addition, the interaction between Pb levels and brood size was also significant, suggesting that Pb had a greater impact on the stress-induced corticosterone of single nestlings than on those of multiple-chick broods. In a previous study, it was reported that single stork nestlings were reared in nests that experienced brood reduction, which suggested lower parental quality (Blas *et al*., 2005). Reduced attendance by young or inexperienced parents may lead singletons to suffer from environmental stressors other than Pb (e.g., a greater exposure to harsh weather conditions). This, in turn, may explain both their higher levels of maximum corticosterone, and the reported stronger relationship between the stress-induced response and Pb. Although similar (i.e., positive) associations between stress response and exposure to Pb have also been reported in rats (Cory Slechta *et al*., 2004) and, more recently, in children exposed pre- and postnatally to low levels of Pb (Gump *et al*., 2008), experimental (Snoeijs *et al*., 2005) and field studies (Eeva *et al*., 2003; Eeva *et al*., 2005) on passerine birds have failed to detect Pb effects on either basal (Snoeijs *et al*., 2005) or stress-induced plasma corticosterone concentrations (Eeva *et al*., 2003; Eeva *et al*., 2005).

Finally, it is important to note that, although the study by Baos et al. (Baos *et al*., 2006) is correlational in nature and comes from a small sample size, it would support the argument that contaminants acting in concert with other stressors may have a greater impact on individuals than the effects elicited by either the contaminants or other stressors acting alone.

SYNTHESIS, STUDY BIAS AND RESEARCH GAPS

Our review reveals that the assessment of adrenocortical parameters (plasma baseline and stress-induced corticosterone concentrations) might be a promising nondestructive biomarker of effect of environmental contaminants in birds. However, it also illustrates that despite a growing number of studies have been published during the last decade, the literature dealing with the impact of pollutants on the stress response in avian species is still very scarce (Table 1), especially when compared with research assessing the impact of chemical contaminants on other physiological systems such as the immune system (Fairbrother *et al*., 2004). Moreover, certain biases and constraints deserving further attention have been identified in our review and are discussed below:

Study Models

A detailed analysis of the Table 1 reveals that the effects of petroleum hydrocarbons and organophosphorous compounds on adrenocortical stress response have been mostly studied on adults or juveniles of poultry species (e.g., chicken, mallard, bobwhite quail) experimentally exposed to variable doses of contaminants in controlled environments. Despite such studies represent a very valuable tool for characterizing the biological action of chemicals and understanding associated toxicity, results are constrained to a particular age segment (i.e., developing individuals are rarely studied), taxa (poultry species), and obtained under captive settings. These conditions imply that extrapolation to wild-bird populations should be done with caution because (1) captivity and domestication can strongly modify HPA function (Romero and Wingfield, 1999), (2) in general, developing birds are more vulnerable to toxicant effects than adults, and this may differentially affect the adrenal stress response, (3) constant dosing regimes (acute exposure to relatively high doses of chemicals) differ from the irregular dietary intake of contaminants that very often characterize environmental exposure (i.e., chronic exposure to low levels of contaminants seems to be the general rule in wild birds, with acute exposure to high doses being restricted to certain hot spots or linked to accidental spills), and (4) the captive study models have limited exposure to real environmental perturbations (e.g., competition, weather inclemency, predation).

Some of these conditions might explain the discrepancies regarding the effects of petroleum hydrocarbons in corticosterone secretion between studies performed in laboratory experiments and those = performed on wild birds (Table 1). Table 1 also reveals a temporal bias in the study of the stress response among classes of contaminants. While the studies on the effects of petroleum and organophosphorous compounds were mostly carried out during the 1980s, the majority of the recent reports deal with environmental exposure to organochlorines and metals. In the latter studies, corticosterone concentration is often measured in developing individuals of altricial or semialtricial species (i.e., nestlings) and within a broader set of biomarkers aimed at assessing the overall health status

of exposed birds. It should be noted that the physiology, diet, and metabolism of young birds is substantially different compared to adults. These differences can modify the patterns of exposure and limit the applicability of data obtained from adult specimens (Burger *et al*., 2003). In general, developing organisms are more vulnerable to toxicant effects (Gochfeld, 1997), and this may differentially affect the adrenal stress response (Gorsline and Holmes, 1982). The developmental stage of particular organs and tissues, and the maturation of endocrine and nervous control can all interact in critical ways to influence the nature of the toxicant effect on the adrenal stress response. Endocrine systems mature at different rates in species showing altricial or precocial modes of development (Blas and Baos, 2008; Scanes and McNabb, 2003). Precocial birds (e.g., mallards, chickens, quails) hatch with sight, covered with down, and are able to thermoregulate, locomote, and feed independently of their parents. Adrenocortical function in response to stressors occurs as early as in 1-day old hatchlings, in contrast with altricial species (e.g., song birds) which hatch almost naked, blind, unable to locomote or thermoregulate, and show little or no response to stress as nestlings (i.e., stress hyporesponsive period). Avian developmental modes vary along a continuum between true precocial and true altricial strategies, and age-related increases in stress-induced corticosterone elevations characterize growing (Blas and Baos, 2008; Blas *et al*., 2006). Thus, the timing of toxicant exposure during development (i.e., in ovo, at hatching, or during growing) may have different effects on the adrenal stress response and depends upon the species developmental mode.

Experimental Protocols

The use of standardized protocols aimed at estimating stress-induced responses such as handling restraint or ACTH challenge have only been incorporated recently, and only among research focused on organochlorine and metal contamination (Table 1). As a consequence, the effects of petroleum hydrocarbons on stress-induced corticosterone levels remain totally unknown (Table 1). With regards to organophosphorous exposure, five studies have tested the effects on stress-induced responses. However, these reports used less conventional experimental stressors like cold temperature or salt water (Rattner and Franson, 1983; Rattner *et al*., 1982a; Rattner *et al*., 1983), increasing the methodological heterogeneity and making it more difficult to establish comparisons among classes of contaminants. This problem also affects studies incorporating standardized capture-restraint protocols and ACTH challenge, because the sampling times following experimental treatments are highly variable, and sometimes corticosterone values are calculated as residuals between the observed levels and those expected from a linear regression with handling time (Eeva *et al*., 2003; Eeva *et al*., 2005). Although basal corticosterone levels constitute an important measure of general stress allowing comparisons within- and between populations, such a static measure is not sufficient to assess adrenocortical function (Norris, 2000), and the collection of additional information on the dynamics of the response to

Table 2 Recommendations for Studying the Adrenocortical Response to Stress in Wild Birds Exposed to Contaminants

- Collect both basal (within 2–3 min after capture) and stress-induced corticosterone concentrations.
- Use standardized protocols (e.g., HR, ACTH injection) to measure stress-induced response.
- Work concurrently on different study populations (to avoid seasonal and interyear variation).
- Measure contaminant exposure on an individual basis (rather than just using population means) both at contaminated and reference sites.
- The use of nondestructive (e.g., blood) or noninvasive (e.g., feathers) methods to estimate adrenocortical function and contaminant exposure is advisable (especially to estimate effects on long-term fitness components, i.e., survival, reproduction).
- Include host (e.g., age, sex, body condition, reproductive stage), ecological, and environmental factors in the statistical analyses.
- Examine how host factors interact with contaminant exposure.
- Consider potential interactions between host factors.
- If work is performed during development, be aware of the developmental mode (within the precocial–altricial spectrum of variation) and the timing of exposure to contaminants.

stress is strongly recommended (Table 2). In fact, the impact of contaminants may only become obvious on stress-induced responses with no effects on basal corticosterone levels, as reported in birds (Baos *et al*., 2006; Bowerman *et al*., 2002; Franceschini *et al*., 2005) and other vertebrates (Norris,, 1999).

An important question deserving special care is whether a given corticosterone measurement constitutes a reliable estimation of baseline levels rather than stress-induced response. Obtaining baseline samples is not always an easy task, and can be especially difficult when animals are captured in the field due to some methodological constraints illustrated in Figure 2. The collection of an initial blood sample shortly following induction of experimental stress is required to assess basal corticosterone concentrations, with time intervals of 2 to 3 minutes following capture, yielding a widely accepted estimation of basal titers (Romero and Reed, 2005). However, after this brief time lag, corticosterone levels rapidly elevate to stress-induced or acute levels over the course of 30 to 60 minutes. A first consideration is that the magnitude of this response depends upon the type of stressor [Fig. 2(A) and (B)]; and therefore, this source of variability handicaps adequate comparisons among studies. A second consideration regards experimental studies where different stressors are sequentially applied to the same individuals [Fig. 2(C)]. Despite acute corticosterone levels may be further elevated following exposure to a second source of stress [e.g., time 4 in Fig. 2(C)], blood samples collected shortly after this time (even within 2–3 minutes, as represented in Fig 2 2(C) by the dashed gray area) should not be considered true basal levels. Avoiding such consideration might lead to an incorrect interpretation of the results, as illustrated in Figure 2(C); individuals from the population exposed to contaminants (gray dots) could be erroneously described as having higher baseline levels and being unable to respond to stress, when in fact they had a lower basal corticosterone and a faster corticosterone elevation compared to control birds (black dots).

This observation is also relevant when comparing the stress response among wild populations, because uncontrolled local perturbations (e.g., inclement weather, parasites) may generate corticosterone elevations prior to the experimental exposure to stress, potentially misleading the interpretation of contaminant-related effects.

Population Versus Individual Approaches

Another important observation regards the use of population mean levels of contaminant exposure rather than individual levels. The observation of adrenocortical differences among populations, even in conjunction with known differences in the presence or amount of certain contaminants, might not be enough to establish reliable associations. Numerous host, ecological, and environmental variables may account for population differences independent of toxic prevalence or exposure. For example, host factors such as age, sex, reproductive status, size and weight, body condition, nutritional status, genetics, and even behavioral interactions may differ among populations and confound results by being correlated with HPA axis activity (Harvey, 1996). In addition, these factors may as well influence the amount and degree of contaminant exposure, uptake, absorption, biokinetics, susceptibility, and toxicity (Peakall and Burger, 2003). Local environmental factors, like weather conditions, parasites, predation attempts, changes in food availability, density of conspecifics, and social competition may also affect the response to stress (Wingfield and Romero, 2001) and should be controlled in order to obtain a reliable estimate of the potential effects of contaminants on the stress response. Recent investigations with white stork nestlings have demonstrated that these factors have the potential to interact with contaminants modulating the stress response of young birds (Baos *et al*., 2006), making, thus very advisable, the assessment of contaminant exposure on an individual- rather than on a population basis.

Fitness Consequences and Extrapolation among Species

Two important questions deserving further attention are whether differences in the stress response associated to contaminant exposure are truly relevant, if they do not translate into a proved impaired/reduced health, reproduction, or survival; and whether demonstration of such effect in one species can be extrapolated to others. To date, the consequences of contaminant-related modulation of stress responses on unequivocal fitness traits remain largely unknown, possibly as a result of the difficulty of maintaining long-term programs of population monitoring in the wild. However, a recent series of investigations on HPA function in wild stork nestlings have provided interesting insights in this regard. In a long-term field study, Blas et al. (Blas *et al*., 2007) found that individuals with a reduced stress response early in life (i.e., as nestlings) had a higher probability of survival and recruitment into the breeding population when adults, providing the first empirical evidence of a link between the physiological response to stress and long-term fitness components in a wild vertebrate. Because a positive relationship between blood Pb levels and the stress response had been previously reported in stork nestlings (Baos *et al*.,

2006), it might be possible that metal contamination exerted some indirect effect on fitness. It is also important to highlight that the association between Pb and adrenocortical stress response reported by Baos et al. (Baos *et al*., 2006) occurred at Pb levels below those considered to cause sublethal effects in birds. Concerning the validity of extrapolation of results among species, it should be noted that the physiological ranges of baseline and stress-induced corticosterone levels shows a strong interspecific variability. As a consequence, obtaining a species-specific reference value is always advisable to determine whether a response falls within the "normal" range of variation. However, rare or endangered species might not be available for sampling, and adverse effects on wildlife may constitute a useful warning signal to anticipate consequences of contaminant exposure on humans. These are strong practical arguments justifying the use of sentinel species, and thus the validity of extrapolation among taxa. An interesting example is a recent study performed on children (Gump *et al*., 2008) reporting a positive relationship between (relatively low) prenatal and postnatal blood Pb levels and adrenocortical response to acute stress, very similar to the results previously reported for wild white storks. From these reports we can conclude that white storks may be considered good sentinels for the detection of potential adverse effects of Pb on human health, providing support to the hypothesis that health effects observed in wildlife and in laboratory animals are predictive of similar health effects in humans (Frame and Dickerson, 2006; Heindel *et al*., 1998). If we accept that white stork nestlings are sentinels for the Pb impact on the adrenal stress response of children, then the emerging data on the long-term consequences of such response on stork fitness deserve serious consideration.

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Compared to other biomarkers of environmental health, the number of publications on adrenal stress response in avian species exposed to environmental contaminants is relatively scarce, especially when restricted to a certain class of chemicals and even more when a particular substance is considered (Table 1). This fact contrasts with the recognition that the adrenal gland is the most frequently observed site of endocrine lesion (Ribelin, 1984) and the unquestioned pivotal role of the HPA axis in maintaining homeostasis. While it is clear that environmental chemicals can modulate the adrenal stress response in avian species, the underlying mechanisms are in most cases poorly understood. Mechanistic information is important to understand and ultimately reduce the uncertainties associated with the ecological risk to wildlife and ultimately to humans caused by EMSs. This type of information demands intense, detailed research.

Laboratory experiments are necessary to explore the relationships between different doses of contaminants and the stress response. One additional concern is the potential interactions among different EMSs on wild animals. When examining the impacts of a mixture of contaminants on endocrine endpoints, is the whole mixture greater than, less than, or equal to the sum of its constituent

parts? This question is particularly intriguing when considering the widespread distribution of many EMSs in the environment that even at low levels may interact with the chemical of primary research interest, potentially leading to unexpected responses and confounding results. Experimental work in this regard should be done. Recent studies in wild storks provide evidence that the concentrations of Pb, positively affecting the stress response, may be lower than the existing threshold reference levels, adding concern to the low-level exposure to many potential EMSs that are widely distributed in the environment. Only within the context of controlled laboratory conditions can individual effects be isolated and causality be established.

Laboratory experiments should nonetheless be combined with field studies. Despite the adrenocortical stress response is not stressor specific, and can be affected by numerous host, ecological, and environmental factors (potentially affecting the toxicity of any given substance), recent findings on several avian species provide evidence that this system can be a good biomarker of environmental health (Baos *et al*., 2006; Mayne *et al*., 2004). The potential application of field models as sentinels justify the need of extended research on the impacts of environmental contaminants on avian HPA function. Research on the many contaminants not tested yet (e.g., brominated flamed retardants) and on those having ubiquitous presence in the environment even at low levels constitutes a study priority that is especially encouraged. Although long-term field studies can be labor-intensive, expensive, and in some cases, logistically very difficult, they are necessary to determine the relevance of contaminant-induced stress responses on long-term fitness components (e.g., survival and reproduction). Recent investigations on white storks support the usefulness of this kind of field studies, which preferentially require long-lived species (e.g., seabirds, raptors) and nondestructive methods of sampling (e.g., blood or feathers). In this regard, a method for the assessment of adrenal function in feather samples has been recently published (Bortolotti *et al*., 2008). This novel approach has the great advantage of being noninvasive because feathers are naturally shed on a regular basis, bird capture is not required, thus reducing sampling effort and avoiding undesirable effects related to wildlife manipulation. On the other hand, feathers have also been used to examine levels of some metals (Burger, 1993), and recent investigations introduce this method as a promising biomonitoring tool for assessing organic pollutants (Jaspers and Covaci, 2006; Van Den Steen and Covaci, 2007). Therefore, the assessment of the impact of environmental contaminants on adrenocortical stress response using feather samples should prove fruitful in the near future.

REFERENCES

WHO/IPCS. Global Assessment of the State-of-the-Science of Endocrine Disruptors. In: Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G, eds. WHO/IPCS/EDC/02.2, Geneva, Switzerland: WHO.

EDSTAC, Final Report, US EPA, Washington, DC, 1998.

Baos R, Blas J, Bortolotti GR, et al. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (*Ciconia ciconia*) exposed to heavy metal and arsenic contamination. Environ Health Perspect 2006; 114:1497–1501.

Becker PH. Biomonitoring with birds. In: Markert BA, Breure AM, Zechmeister HG, eds. Bioindicators and Biomonitors, Oxford, UK: Elsevier; 2003:677–736.

Blas J, Baos R. Stress in the nest: Causes and consequences of adrenocortical secretion in developing birds. In: Capaldo A, ed. Recent Advances in Non-mammalian Adrenal Gland Research, Research SignPost, 2008. For a review. http://www.trnres. com/ **AQ2**

Blas J, Baos R, Bortolotti GR, et al. A multi-tier approach to identifying environmental stress in altricial nestling birds. Functional Ecology 2005; 19:315–322.

Blas J, Baos R, Bortolotti GR, et al. Age-related variation in the adrenocortical response to stress in nestling white storks (*Ciconia ciconia*) supports the developmental hypothesis. Gen Comp Endocrinol 2006; 148:172–180.

Blas J, Bortolotti GR, Tella JL, et al. Stress response during development predicts fitness in a wild, long-lived vertebrate. Proc Natl Acad Sci USA 2007; 104:8880–8884.

Borga K, Gabrielsen GW, Skaare JU. Biomagnification of organochlorines along a Barents Sea food chain. Environ Pollut 2001; 113:187–198.

Bortolotti GR, Marchant TA, Blas J, et al. (2008). Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Functional Ecology doi: 10.1111/j.1365–2435.2008.01387.x.

Bowerman WW, Mehne CJ, Best DA, et al. Adrenal corticotropin hormone and nestling Bald eagle corticosterone levels. Bull Environ Contam Toxicol 2002; 68:355–360.

Burger J, Diaz-Barriga F, Marafante E, et al. Methodologies to examine the importance of host factors in bioavailability of metals. Ecotoxicol Environ Saf 2003; 56:20–31.

Burger J. Metals in avian feathers: Bioindicators of environmental pollution. Rev Environ Toxicol 1993; 5:203–311.

Burrell GA, Seibert FM. Gases found in coal mines. Miners' Circular 14. Bureau of Mines. Department of the Interior, Washington, DC; 1916

Cabezas S, Blas J, Marchant TA, et al. Physiological stress levels predict survival probabilities in translocated wild rabbits. Horm Behav 2006; 51:313–320.

Capdevielle MC, Carsia RV, Scanes CG. Effect of acid or aluminum on growth and adrenal function in young chickens. Gen Comp Endocrinol 1996; 103:54–59.

Civen M, Brown CB. The effect of organophosphate insecticides on adrenal corticosterone formation Pestic Biochem Physiol 1974; 4:254–259.

Civen M, Lifrack E, Brown CB. Studies on the mechanism of inhibition of adrenal steroidogenesis by organophosphate and carbamate compounds. Pestic Biochem Physiol 1977; 7:169–182.

Colby HD, Longhurst PA. Toxicology of the adrenal gland. In: Atterwill CK, Flack JD, eds. Endocrine Toxicology. Cambridge, UK: Cambridge University Press; 1996:243–281.

Conney AH, Welch RM, Kuntzman R, et al. Effect of pesticides on drug and steroid metabolism. Clin Pharmacol Ther 1971; 8:2–10.

Cory Slechta DA, Virgolini MB, Thiruchelvam M, et al. Maternal stress modulates the effects of developmental lead exposure. Environ Health Perspect 2004; 112:717–730.

Dallman MF. Stress by any other name? Horm Behav 2003; 43:18–20.

DeRosa C, Richter P, Pohl H, et al. Environmental exposure that affects the endocrine system: Public health implications. J Toxicol Environml Health Part B 1998; 1: 3–26.

Di Giulio RT, Scanlon PF. Sublethal effects of cadmium ingestion on mallard ducks. Arch Environm Contam Toxicol 1984; 13:765–771.

Di Giulio RT, Scanlon PF. Effects of cadmium ingestion and food restriction on energy metabolism and tissue metal concentrations in mallard ducks (*Anas platyrhynchos*). Environ Res 1985; 37:433–444.

Eeva T, Lehikoinen E, Nikinmaa M. Pollution-induced nutritional stress in birds: An experimental study of direct and indirect effects. Ecol Appl 2003; 13:1242–1249.

Eeva T, Hasselquist D, Langefors A, et al. Pollution related effects on immune function and stress in a free-living population of pied flycatcher Ficedula hypoleuca. J Avian Biol 2005; 36:405–412.

Fairbrother A, Smits J, Grasman KA. Avian immunotoxicology. J Toxicol Environ Health Part B 2004; 7:105–137.

Fleming WJ, Heinz GH, Franson JC. Toxicity of Abate 4E (temephos) in mallard ducklings and the influence of cold. Environ Toxicol Chem 1985; 4:193–199.

Foil L, Satterlee D, Jacobs-Perry L. Increased serum corticosterone levels in triorthotolyl phosphate-treated chickens. Bull Environ Contam Toxicol 1985; 34:780–786.

Forbes VE, Palmqvist A, Bach L. The use and misuse of biomarkers in ecotoxicology. Environ Toxicol Chem 2006; 25:272–280.

Fowler GS, Wingfield JC, Boersma PD. Hormonal and reproductive effects of low levels of petroleum fouling in Magellanic penguins (*Spheniscus magellanicus*). Auk 1995; 112:382–389.

Fowles JR, Fairbrother A, Trust KA, et al. Effects of aroclor 1254 on the thyroid gland, immune function, and hepatic cytochrome P450 activity in mallards. Environ Res 1997; 75:119–129.

Fox GA. Practical causal inference for ecoepidemiologists. J Toxicol Environ Health 1991; 33:359–373.

Fox GA. Wildlife as sentinels of human health effects in the Great Lakes-St. Lawrence Basin. Environ Health Perspect 2001; 109(Suppl. 6):853–861.

Frame L, Dickerson RL. Fish and wildlife as sentinels of environmental contamination. In: Norris DO, Carr JA. eds. Endocrine Disruption: Biological Bases for Health Effects in Wildlife and Humans. NY: Oxford University Press; 2006:202–222.

Franceschini MD, Custer CM, Custer TW, et al. Corticosterone stress response in tree swallows (*Tachycineta bicolor*) nesting near a PCB-contaminated river. Integr Comp Biol 2005; 45(6), 1132.

Freeman BM, Manning ACC. Re-establishment of the stress response in gallus domesticus after hatching. Comp Biochem Physiol Part A 1984; 78:267–270.

Furness RW. Birds as monitors of pollutants. In: Furness RW, Greenwood JJD, eds. Birds as Monitors of Environmental Change. London, UK: Chapman & Hall, 1993:87–143.

Gervais JA, Anthony RG. Chronic organochlorine contaminants, environmental variability, and the demographics of a burrowing owl population. Ecol Appl 2003; 13:1250–1262.

Gochfeld M. Factors influencing susceptibility to metals. Environl Health Perspect 1997; 105(Suppl.4):817–822.

Gorsline J, Holmes WN. Effects of petroleum on adrenocortical activity and on hepatic naphthalene-metabolizing activity in mallard ducks. Arch Environ Contam Toxicol 1981; 10:765–777.

Gorsline J, Holmes WN. Suppression of adrenocortical activity in mallard ducks exposed to petroleum-contaminated food. Arch Environ Contam Toxicol 1982; 11:497–502.

Gorsline J, Holmes WN. Adrenocortical function and hepatic naphthalene metabolism in mallard ducks (*Anas platyrhynchos*) consuming petroleum distillates. Environ Res 1982; 28:139–146.

Gorsline J, Holmes WN. Variations with age in the adrenocortical responses of mallard ducks (*Anas platyrhynchos*) consuming petroleum-contaminated food. Bull Environ Contam Toxicol 1982; 29:146–152.

Gorslineet al. 1981. **AQ1**

Gorsline J. Impairment of adrenocortical function by ingested petroleum hydrocarbons. J Steroid Biochem Mol Biol 1984; 20(6B):1546.

Grasman KA, Scanlon PF, Fox GA. Reproductive and physiological effects of environmental contaminants in fish-eating birds of the Great Lakes: A review of historical trends. Environ Monit Assess 1998; 53:117–145.

Gross WB. Effect of adrenal blocking chemicals on the responses of chickens and turkeys to environmental stressors and ACTH. Avian Physiol 1990; 19:295–304.

Guillette LJ Jr, Kools SAE, Gunderson MP, et al. DDT and its analogues: New insights into their endocrine-disrupting effects on wildlife. In: Norris DO, Carr JA, eds, Endocrine Disruption: Biological Bases for Health Effects in Wildlife and Humans NY: Oxford University Press; 2006:332–355.

Gump BB, Stewart P, Reihman J, et al. Low-level prenatal and postnatal blood lead exposure and adrenocortical responses to acute stress in children. Environ Health Perspect 2008; 116:249–255.

Hadley AJ, Flack JD, Buckingham JC. Modulation of corticotrophin release in vitro by methylxanthines and adenosine analogues. Br J Pharmacol 1990; 100(Suppl): 337.

Harvey S, Klandorf H, Phillips JG. Reproductive performance and endocrine responses to ingested petroleum in domestic ducks (*Anas platyrhynchos*). Gen Comp Endocrinol 1981; 45:372–380.

Harvey PW. The Adrenal in Toxicology: Target Organ and Modulator of Toxicity. London, UK: Taylor & Francis; 1996

Heath JA, Frederick PC. Relationships among mercury concentrations, hormones, and nesting effort of White ibises (*Eudocimus albus*) in the Florida Everglades. Auk 2005; 122:255–267.

Heindel JJ, Collman GW, Suk WA. Endocrine disruptors: Extrapolation from wildlife to human effects. In: Kendall RJ, Dickerson RL, Giesy J, Suk W, eds. Principles and Processes for Evaluating endocrine Disruption in Wildlife. Pensacola, FL: SETAC Press; 1998:369–430.

Heinz GH. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. JWildl Manage 1979; 43:394–401.

Hickey JJ, Anderson DW. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 1968; 162:271–273.

Hinson JP, Raven PW. Effects of endocrine-disrupting chemicals on adrenal function. Best Pract Res Clin Endocrinol Metab 2006; 20:111–120.

Jönsson CJ, Lund BO, Brunström B, et al. Toxicity and irreversible binding of two DDT metabolites—3-methylsulfonyl-DDE and *o,p′*-DDD—in adrenal interrenal cells in birds. Environ Toxicol Chem 1994; 13:1303–1310.

Jacobs LF, Wingfield JC. Endocrine control of life-cycle stages: A constraint on response to the environment? Condor 2000; 102:35–51.

Jaspers VLB, Voorspoels S, Covaci A, et al. Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? Biol Lett 2006; 2:283–285.

Joseph J, Ramachandran AV. Effect of exogenous dexamethasone and corticosterone on weight gain and organ growth in post-hatched white leghorn chicks. Indian J Exp Biol 1993; 31:858–860.

Kitaysky AS, Wingfield JC, Piatt JF. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged kittiwakes. Funct Ecol 1999; 13:577–584.

Kitaysky AS, Kitaiskaia EV, Piatt JF, et al. Benefits and costs of increased levels of corticosterone in seabird chicks. Horm Behavr 2003; 43:140–149.

Kitaysky AS, Kitaiskaia EV, Wingfield JC,et al. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in Red-legged kittiwake chicks. J Comp Physiol Part B 2001; 171:701–709.

Kushlan JA. Colonial waterbirds as bioindicators of environmental change. Colonial Waterbirds 1993; 16:223–251.

LeBlanc GA. Are environmental sentinels signaling? Environmental Health Perspectives 1995; 103:888–890.

Lorenzen A, Moon TW, Kennedy SW, et al. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes Herring gull embryos. Environ Health Perspect 1999; 107:179–186.

Love OP, Shutt LJ, Silfies JS, et al. Repeated restraint and sampling results in reduced corticosterone levels in developing and adult captive American kestrels (*Falco sparverius*). Physiol Biochem Zool 2003a; 76:753–761.

Love OP, Shutt LJ, Silfies JS, et al. Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). Ecotoxicology 2003b; 12:199–208.

Lower WR, Kendall RJ. Sentinel species and sentinel bioassays. In: McCarthy JF, Shugart LR, eds. Biomarkers of Environmental Contamination. Chelsea, MI: Lewis Publishers; 1992:309–331.

Lund B. In vitro adrenal bioactivation and effects on steroid metabolism of DDT, PCBs, and their metabolites in the Gray seal (*Halichoerus gryus*). Environ Toxicol Chem 1994; 13:911–917.

Marra PP, Holberton RL. Corticosterone levels as indicators of habitat quality: Effects of habitat segregation in a migratory bird during the non-breeding season. Oecologia 1998; 116:284–292.

Martin II LB, Gilliam J, Han P, et al. Corticosterone supresses cutaneous immune function in temperate but no tropical House sparrows, *Passer domesticus*. Gen Comp Endocrinol 2005; 140:126–135.

Martinovic B, Lean DL, Bishop CA, et al. Health of Tree swallows (*Tachycineta bicolor*) nestlings exposed to chlorinated hydrocarbons in the St. Lawrence River Basin. Part II. Basal and stress plasma corticosterone concentrations. J Toxicol Environ Health Part A 2003; 66:2015–2029.

Mayne GJ, Martin PA, Bishop CA, et al. Stress and immune responses of nestling Tree swallows (*Tachycineta bicolor*) and Eastern bluebirds (*Sialis sialis*) exposed to nonpersistent pesticides and *p*,*p*′-dichlorodiphenyldichloroethylene in apple orchards of southern Ontario, Canada. Environ Toxicol Chem 2004; 23:2930–2940.

McCarty JP. Use of tree swallows in studies of environmental stress. Rev Toxicol 2002; 4:61–104.

McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Horm Behav 2003; 43:2–15.

Miller DS, Peakall DB, Kinter WB. Ingestion of crude oil: Sublethal effects in Herring gull chicks. Science 1978; 199:315–317.

Mineau P, Fletcher MR, Glaser LC, et al. Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, U. S. and U. K. J Raptor Res 1999; 33:1–37.

Mullner A, Linsenmair KE, Wikelski M. Exposure to ecotourism reduces survival and affects stress response in Hoatzin chicks (*Opisthocomus hoazin*). Biol Conserv 2004; 118:549–558.

Newcomer WS. Adrenal and blood A^4-?-keto-corticosteroids following various treatments in the chick. Am J Physiol 1959; 196:276–278.

Norris DO. Endocrine disruptors of the stress axis in Natural Populations: How can we tell? Am Zool 2000; 40:393–401.

Norris, 1999. **AQ3**

Ottinger MA, Abdelnabi MA, Henry P, et al. Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail. Horm Behav 2001; 40:234–247.

Peakall DB, Burger J. Methodologies for assessing exposure to metals: Speciation, bioavailability of metals, and ecological host factors. Ecotoxicol Environ Saf 2003; 56:110–121.

Peakall DB, Tremblay J, Kinter WB, et al. Endocrine dysfunction in seabirds caused by ingested oil. Environ Res 1981; 24:6–14.

Peakall DB. Pesticide-induced enzyme breakdown of steroids in birds. Nature 1967; 216:505.

Phillips B, Harrison P. Overview of the endocrine disrupters issue. In: Hester RE, Harrison RM, eds. Endocrine Disrupting Chemicals. Cambridge, UK: The Royal Society of Chemistry, 1999:1–25.

Pottinger TG. Interactions of endocrine disrupting chemicals with stress response in wildlife. Pure Appl Chem 2003; 75:2321–2333.

Quinn MJ Jr., Summitt CL, Ottinger MA. Consequences of in ovo exposure to p,p′-DDE on reproductive development and function in Japanese quail. Horm Behav 2008; 53:249–253

Ramade F, Baylé JD. Evaluation de l'activité hypophyso-corticosurrénalienne basale et de sa réponse au stress au cours de la période précédant et suivant l'éclosion chez la pigeon. J Physiol Paris 1980; 76:283–287.

Ratcliffe DA. Changes attributed to pesticides in egg breakage frequency and eggshell thickness in some British birds. J Appl Ecol 1970; 7:67–115.

Rattner BA, Franson JC. Methyl parathion and fenvalerate toxicity in American kestrels: Acute physiological responses and effects of cold. Can JPhysiol Pharmacol 1983; 62:787–792.

Rattner BA, Sileo L, Scanes CG. Hormonal responses and tolerance to cold of female quail following parathion ingestion. Pestic Biochem Physiol 1982a; 18:132–138.

Rattner BA, Sileo L, Scanes CG. Oviposition and the plasma concentrations of LH, progesterone and corticosterone in Bobwhite quail (*Colinus virginianus*) fed parathion. J Reprod Fertil 1982b; 66:147–155.

Rattner BA, Fleming WJ, Murray HC. Osmoregulatory function in ducks following ingestion of the organophosphorus insecticide fenthion. Pestic Biochem Physiol 1983; 20:246–255.

Rattner BA. Tolerance of adult mallards to subacute ingestion of crude petroleum oil. Toxicol Lett 1981; 8:337–342.

Rattner BA, Eastin, WC Jr. Plasma corticosterone and thyroxine concentrations during chronic ingestion of crude oil in mallard ducks (*Anas platyrhynchos*). Comp Biochem Physiol Part C 1981; 68:103–107.

Rehulka J, Kraus M. Regulation of corticosterone metabolism in liver cell fractions in young and adult rats: Cofactor requirements, effects of stress and phenobarbital treatment. Physiol Bohemoslov 1987; 36:21–32.

Ribelin WE. The effects of drugs and chemicals upon the structure of the adrenal gland. Fundam Appl Toxicol 1984; 4:105–119.

Romero LM, Reed JM. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? Comp Biochem Physiol Part A 2005; 140;73–79.

Romero LM, Wingfield JC. Alterations in hypothalamic-pituitary-adrenal function associated with captivity in Gambel's white-crowned sparrow (*Zonotrichia leucophris gambelii*). Comp Biochem Physiol Part B 1999; 122:13–20.

Romero LM. Physiological stress in ecology: Lessons from biomedical research. Trends Ecol Evol 2004; 19:249–255.

Romijn CAFM, Grau R, Guth JA, et al. The use of Japanese and Bobwhite quail as indicator species in avian toxicity test. Chemosphere 1995; 30:1033–1040.

Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000; 21:55–89.

Sapolsky RM. Neuroendocrinology of the stress-response. In: Becker, B., Breedlove SM, Crews D, eds. Behavioural Endocrinology. Cambridge, MA: MIT Press; 1992:287–324.

Scanes CG, McNabb FMA. Avian models for research in toxicology and endocrine disruption. Avian Poult Biol Rev 2003; 14:21–52.

Schwabe CW(). Animal monitors of the environment. In Veterinary Medicine and Human Health. Baltimore, MD: Williams & Wilkins; 1984:562–578

Scollon EJ, Carr JA, Cobb GP. The effect of flight, fasting, and *p,p*'-DDT on thyroid hormones and corticosterone in Gambel's white crowned sparrow, Zonotrichia leucophrys gambelli. Comp Biochem Physiol Part C 2004; 137:179–189.

Selye H. A syndrome produced by diverse nocuous agents. Nature 1936; 138:32.

Sheffield SR, Matter JM, Rattner BA, et al. Fish and wildlife species as sentinels for environmental endocrine disrupters. In: Kendall R J, Dickerson RL, Giesy J, Suk W, eds. Principles and Processes for Evaluating Endocrine Disruption in Wildlife. Pensacola, FL: SETAC Press; 1998:369–430.

Snoeijs T, Dauwe T, Pinxten R, et al. The combined effect of lead exposure and high or low dietary calcium on health and immunocompetence in the Zebra finch (*Taeniopygia guttata*). Environ Pollut 2005; 134:123–132.

Spindel E, Griffiths L, Wurtman RJ. Neuroendocrine effects of caffeine. II. Effects on thyrotropin and corticosterone secretion. J Pharmacol Exp Ther 1983; 225:346–350.

Srebocan V, Pompe-Gotal J, Adamovic V, et al. Effect of technical grade DDT and *p,p′*-DDT on adrenocortical function in chicks. Poult Sci 1971; 50:1271–1278.

Stahl, RG Jr. Can mammalian and non-mammalian "sentinel species" data be used to evaluate the human health application of environmental contaminants? Human and Ecological Risk Assessment 1997; 3:329–335.

Suorsa P, Huhta E, Nikula A, et al. Forest management is associated with physiological stress in an old-growth forest passerine. Proc R Soc Lond B Biol Sci B 2003; 270:963–969.

Van Den Steen E, Covaci A, Jaspers VLB, et al. Experimental evaluation of the usefulness of feathers as a non-destructive biomonitor for polychlorinated biphenyls (PCBs) using silastic implants as a novel method of exposure. Environ Int 2007; 33:257–264.

Vos JG, Dybing E, Greim HA, et al. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Criti Rev Toxicol 2000; 30:71–133.

Waid JS. PCBs in the environment (Vol. I–III). Cleveland, OH: CRC Press, 1985–1987.

Walker BG, Dee Boersma P, Wingfield JC. Field endocrinology and conservation biology. Integr Comp Biol 2005; 45:12–18.

Walker BG, Boersma PD, Wingfield JC. Physiological and behavioral differences in Magellanic penguin chicks in undisturbed and tourist-visited locations of a colony. Conserv Biol 2005; 19:1571–1577.

Walsberg GE. How useful is energy balance as an overall index of stress in animals? Horm Behav 2003; 43:16–17.

Wasser SK, Bevis K, King G, et al. Noninvasive physiological measures of disturbance in the Northern spotted owl. Conserv Biol 1997; 11:1019–1022.

Wayland M, Smits JEG, Gilchrist HG, et al. Biomarker responses in nesting, common eiders in the Canadian Arctic in relation to tissue cadmium, mercury and selenium concentrations. Ecotoxicology 2003: 12:225–237.

Wayland M, Gilchrist HG, Marchant T, et al. Immune function, stress response, and body condition in Arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. Environ Res 2002; 90:47–60.

Wingfield JC, Ramenofsky M. Hormones and the behavioral ecology of stress. In: Balm PHN, ed. Stress Physiology in Animals. Sheffield, UK: Sheffield Academic Press; 1999:1–51.

Wingfield JC, Romero LM. Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS, ed). Handbook of Physiology. Section 7: The Endocrine System, Vol. 4: Coping with the Environment: Neural and Endocrine Mechanisms. Oxford, UK: Oxford University Press; 2001:211–236.

Wingfield JC. Control of behavioural strategies for capricious environments. Anim Behav 2003; 66:807–815.

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