

# Factors Considered in Using Birds for Evaluating Endocrine-disrupting Chemicals

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## Abstract

Documented effects on fish and wildlife populations, coupled with evidence from human poisonings, epidemiology, and experimental toxicology, led to the formation of the Endocrine Disruptor Screening Program within the US Environmental Protection Agency. The main objectives of the program are to validate and implement the screens and tests that have been proposed for evaluating possible endocrine-disrupting activity of chemicals. An avian two-generation test is one of the recommended higher tier tests currently undergoing prevalidation. The advantages and disadvantages of the two species of quail considered as candidates, the northern bobwhite (*Colinus virginianus*) and the Japanese quail (*Coturnix japonica*), are described as well as the basis for final selection of the Japanese quail. Among the numerous considerations necessary for ultimately optimizing a two-generation test method using birds, the following key factors are discussed: the number of birds used in the test, when to begin exposure of the P generation, selection and exposure of the F1 generation, and endpoints.

**Key Words:** avian models; development; endocrine disruptors; growth; reproduction; testing; toxicants

## Introduction

Concern that synthetic chemicals can mimic or otherwise interfere with natural hormones has existed since the early 1970s (Bitman and Cecil 1970; Bitman et al. 1968; Hertz 1985; McLachlan 1980; Nelson et al. 1978). Public awareness of these concerns increased in the 1990s largely through the efforts of Theo Colborn and the World Wildlife Fund (Colborn and Clement 1992; Colborn et al. 1993, 1996). This increased public awareness led to the passage of two laws in 1996: the Food Quality Protection Act (FQPA<sup>1</sup>) and amendments to the Safe Drinking

Water Act (SWDA<sup>1</sup>). Specifically, the US Environmental Protection Agency (EPA<sup>1</sup>) was charged to evaluate substances found in food or drinking water sources to determine whether they cause estrogenic or other endocrine activity (Federal Register 1998a,b).

Endocrine disruption as a phenomenon has been critically reviewed by several authors, notably by Crisp and colleagues (1998), the National Research Council (NRC 1999), Damstra and coworkers (2002), and Ottinger and vom Saal (2002). A common conclusion from these reviews is that the evidence for disturbances to the endocrine systems of certain fish and wildlife by chemicals that contaminate their habitats is compelling.

Birds have been particularly important among the wildlife species that have been observed in an effort to understand the deleterious effects of endocrine disruption. Rachel Carson's *Silent Spring* (Carson 1962) might be regarded as the first to bring attention to the sensitive nature of birds to the deleterious effects of toxic contaminants. Damstra and coauthors (2002) discuss the unique characteristics of birds that results in their particular vulnerability to potential endocrine-disrupting chemicals (EDCs<sup>1</sup>). Briefly, characteristics of birds that have a profound influence on the toxicological and toxicokinetic consequence of an EDC exposure include the following: high rates of food consumption, high metabolic rates, periods of starvation that mobilize lipid reserves, hormone-dependent behaviors, developmental strategies, and control of sexual differentiation. Ultimately, the biological fitness of a bird can be dramatically affected by very subtle changes in the normal functioning and balance of its endocrine system. Scanes and McNabb (2003) present an excellent review of avian toxicology as related to the effects of common toxicants that disrupt endocrine system function and control of reproduction, growth, development, and other processes.

For almost all toxic chemicals, the toxic action or stress exerted on an organism is moderated by endocrine and/or immune processes that exist to maintain homeostasis (Crisp et al. 1998). As a result, it is difficult to differentiate between a direct disruption of an endocrine process or whether the disruption is a consequence of another systemic stress. The ability to make this distinction is important for characterizing a particular chemical as an "endocrine disruptor"; however, the distinction is less important if the goal is only to evaluate the possible adverse consequence similar to an effect of a hormone action or interference with that action. Pursuant to this latter goal, the FQPA stipulates that the

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<sup>1</sup>Abbreviations used in this article: EDSP, Endocrine Disruptor Screening Program; EDSTAC, Endocrine Disruptor Screening and Testing Advisory Committee; EPA, Environmental Protection Agency; FQPA, Food Quality Protection Act; SWDA, Safe Water Drinking Act.

EPA “develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate” (FQPA 1996). In response to this mandate, the EPA created the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC<sup>1</sup>), a Federal advisory committee, to assist EPA in designing an Endocrine Disruptor Screening Program (EDSP<sup>1</sup>). EDSTAC recommended the EPA’s expansion of the scope of the EDSP to include androgen and thyroid hormone pathways, effects on wildlife, and an expanded universe of chemicals (EDSTAC 1998).

The purpose of this article is to promote consideration of the role birds play in the EPA EDSP, and to describe important aspects of their test methodology. Many of the factors described in this article have been discussed by an avian expert group convened by the Organisation for Economic Cooperation and Development (Bennett et al. 2001).

## Endocrine Disruptor Screening Program

In an effort to respond to the mandate provided in FQPA and SDWA, the EPA established the EDSTAC, to provide recommendations regarding a strategy for developing an appropriate screening and testing paradigm. Adhering closely to the recommendations made by the EDSTAC in its final report (EDSTAC 1998), the EPA proposed the EDSP as a two-tiered approach, to consist of a combination of the following: *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1); and a set of long-term *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants (Federal Register 1998a,b).

A joint panel of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel and the EPA Science Advisory Board reviewed and largely concurred with the proposed approach of the EDSP in May 1999. Gray and colleagues (1997), EDSTAC (1998), and the National Research Council (1999) all concluded that a tiered approach relying on a combination of *in vivo* and *in vitro* screens for Tier 1 was scientifically reasonable. It was also consistently held that the individual screens and tests should be validated. Validation and peer review are prerequisites in the development and acceptance of test guidelines for regulatory use (ICCVAM 2000).

## Considerations for Screening Assays

Birds were not recommended for inclusion in the first-level screening assays based on the expert advisory panel’s assumption that the range of *in vitro* and *in vivo* assays included would be sufficiently comprehensive to detect any activity likely to occur also in birds (EDSTAC 1998). The

panel’s rationale emphasized that in available studies, a compound found to be active in a bird endocrine process (however unique it may be to birds) is found to be active also in a mammal or fish endocrine process. Mammals and fish were viewed as representative “book ends” of the vertebrate taxa and should be sufficiently adequate to detect a chemical with the potential to interfere with an endocrine process in any other vertebrate, including birds. There was also a desire to economize in the screening battery, and to reduce any unnecessary testing and duplication to save animals and other resources.

The EDSTAC plan is to assess the validity of the assumption that the assays selected for Tier 1 are both sufficient and necessary in a “battery” validation after the individual assays that comprise the Tier 1 screening battery have each been thoroughly validated (Federal Register 1998b). Details of the concepts and design parameters for Tier 1 screening are available (EDSTAC 1998).

## Considerations for Testing

Birds, although not part of the Tier 1 screening battery, are an important taxa group included in the Tier 2 tests. The following characteristics of birds are among the many that are different from other vertebrate taxa: they are homeothermic and oviparous, and their sexual differentiation is controlled differently (e.g., males are the homogametic sex). The screening battery may be sufficient to detect the possibility that a chemical can interfere with a vertebrate endocrine system, including that of birds; however, tests in other vertebrate animals would have little value in predicting the relationship of dose with potential adverse consequences in birds.

Ideally, a Tier 2 test will accomplish the following:

- Establish exposure, concentrations, timing, and effect relationships;
- Be sensitive and specific;
- Assess relevant endpoints; and
- Provide information for determining whether effects are a primary or secondary disturbance of endocrine function.

Tier 2 testing is the final phase of the EDSP and is intended to provide more detailed information about the endocrine disruption activity of a chemical and the adverse consequence relevant to the taxa of interest. To fulfill this purpose, tests must expose animals in a series of concentrations (dose levels) through all critical life stages. Such tests are logistically challenging but necessary to ensure that any deleterious effect associated with a putative endocrine disrupting compound can be quantitatively assessed.

## Avian Two-generation Toxicity Test

The four key life stages of birds wherein endocrine-mediated processes are most likely subject to disruption

include the following: (1) in ovo (embryonic development); (2) offspring (juvenile growth); (3) subadults (sexual maturation); and (4) adults (sexual reproduction). To ensure that all of these life stages are exposed to potential endocrine-active materials and that observations of any deleterious response can be made, a fully comprehensive test must encompass two generations.

Embryonic development for oviparous species like birds depends in large part on factors transferred to the egg from maternal deposition. These transferred factors include not only a contaminant, for instance, but also factors such as hormones and proteins, which are required to support and direct the development of the embryo. The embryo experiences a function of maternal exposure and deposition, and the full consequence of this experience may not be manifest until the embryo (F1 generation) fully develops, sexually differentiates, reaches maturity, and reproduces. Thus, an EDC that affects the transfer of estrogen into the egg, for instance, potentially affects the sexual and/or behavioral differentiation of the offspring, which in turn affects its reproductive viability. Such effects may be seen in the sex ratio of offspring, the fertility of these offspring at maturity, or the number of eggs produced by these offspring.

Because two generations of exposure and observation are required for a test to characterize potential endocrine-mediated effects adequately, the choice of a suitable test species is somewhat limited. Two species of quail that have been considered to be the most likely candidates are the northern bobwhite (*Colinus virginianus*) and the Japanese quail (*Coturnix japonica*). Both species are fairly representative of terrestrial birds and are already established models for assessing both acute and reproductive effects of pesticides and other chemicals in wild birds (ASTM 1990; EPA 1982, 1985; OECD 1993). Unlike most birds, both species breed well under laboratory conditions and are photoresponsive in their breeding cycle. For this reason, they can be brought into their egg-laying cycles artificially and can produce eggs almost indefinitely in extended light conditions.

Both species are also precocial, meaning that chicks are hatched in a relatively mature state. This characteristic is in contrast to the altricial species, which are hatched in an earlier state of development and require more parental care as they complete development ex ovo. Precocial species allow for much more logistical ease in long-term laboratory management, although they may not undergo certain subtle developmental changes that manifest only in altricial species.

Of the two species, the Japanese quail is considered the most suitable for a two-generation test primarily due to its rapid incubation and maturation time and consistently high egg production. This combination of factors allows for completion of a full two-generation test in approximately 30 wk, less than half the time of the 70 wk expected with the bobwhite. Several other aspects of the Japanese quail also argue strongly in its favor, including the following: (1) they produce a large number of eggs per hen for sampling egg quality and production; (2) males possess a cloacal gland

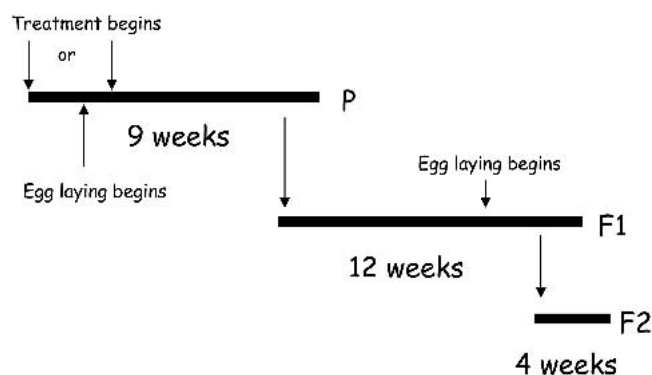
that can be monitored as an indirect measure of gonadal development, reproductive fitness, and sexual maturation; and (3) the species has been used extensively in molecular, biochemical, and endocrine research, and its behavioral patterns have been well characterized (Hutchinson et al. 2000; Ottinger and Brinkley 1978, 1979a,b).

Existing avian reproduction toxicity tests (ASTM 1990; OECD 1993) expose adults prior to the onset of maturation and egg laying and continue exposure through the egg-laying period. Offspring are provided an untreated diet and monitored for 14 days after hatch. These offspring would, however, be exposed to residues from maternal deposition in their early development in ovo. This test is satisfactory for assessing the immediate impact on fertility and egg production and on the short-term viability and sensitivity of offspring. However, the test does not address possible effects on the sexual differentiation (sex ratio) of offspring produced or on the reproductive viability (behaviorally and physiologically) of these offspring. To collect information on these latter potential effects, the test method must be extended to accommodate two generations.

Therefore, a two-generation test is designed to expose to a suspected chemical an initial generation of birds (parental generation or P) that are allowed to reproduce and result in a second generation of birds (first filial generation or F1). The F1s are allowed to mature and reproduce, which results in production of a third generation (second filial generation or F2). It should be noted, however, that several technical and logistical elements that influence the selection of an optimal experimental design must be considered carefully. Key elements to note are the number of birds used in the test, when to begin exposure of the P generation, selection and exposure of the F1 generation, and endpoints to be included. Figure 1 provides a schematic overview of a two-generation test performed with Japanese quail.

## Number of Birds

One feature that makes Japanese quail attractive as a test species for these long-term tests is their ability to produce



**Figure 1** Schematic overview of a Japanese quail two-generation test.

large numbers of eggs consistently. This prolific egg production brings with it logistical constraints and ethical issues with the potential large numbers of animals that may be utilized. The experimental design of the study must balance statistical power for quantifying effects with using only a minimum number of animals to provide reliable and reproducible results. Birds, like all living organisms, have natural variability in responses that must be addressed. The statistical power for reproduction tests with quail have been reviewed elsewhere (Bennett and Bennett 1990; Bennett and Ganio 1991; Collins 1994; Mineau et al. 1994; Springer and Collins 1999). In the Collins review (1994), the author indicates that 80% power requires 74 pens (pairs of birds) to detect a 20% reduction in the number of eggs laid or 27 pens to detect a 20% decline in number of hatchlings per eggs incubated. By using proven breeders, Springer and Collins (1999) determined that as few as 12 pens will suffice if one also includes a covariate.

Because the number of birds used in a two-generation test is, to some extent, a function of how many pairs are used at initiation in the P generation, it is critical to ascertain for the range of endpoints to be included in this expanded test paradigm the number that will provide consistently reliable results. Ultimately, the number of eggs collected and incubated to hatch for the F1 generation and additionally for the F2 generation will determine the total number of birds used in a given experiment. By focusing analysis on the time of peak egg production, which in Japanese quail can be very consistent, and limiting the number of eggs to be hatched from selected cohorts in both the F1 and F2 generation, it is believed that the total number of birds utilized in this two-generation test may be no more and likely substantially less than would be used in an avian reproduction test performed under existing test guidelines (ASTM 1990; OECD 1993). The number of birds ultimately used should be determined after selecting the power, significance level, and size of the effect to be detected based on the observed population variability of the individual endpoints being studied (Dell et al. 2001).

## Exposure of P Generation

Existing avian reproduction tests begin exposures before the onset of egg laying. However, even control pairs are often infertile, and the effects of test chemicals are often obscured by this normal variability. For this reason, consideration has been given to beginning exposure with pairs that have proven fertile egg-laying ability. Significant reproductive effects have been reported from only short-term exposures to a toxicant (Bennett and Bennett 1990; Bennett et al. 1990; Rattner et al. 1982; Stromborg 1981, 1986). As stated above, Springer and Collins (1999) demonstrated that using proven breeders can substantially reduce the number of pairs needed. However, the question of EDCs remains with respect to whether pre-egg-laying exposure can affect individual maturation and/or the quality and extent of maternal

transfer to the eggs. Certain effects cannot be determined in the P generation when exposure occurs after achievement of reproductive maturity. In wild bird populations, alteration in the time to sexual maturation is one such endocrine-related effect that has serious implications to reproductive success (Yoshimura et al. 2000). It is also necessary to account for the potential of a compound to bioaccumulate, and to consider whether exposure must occur before egg laying to allow sufficient time for equilibrium to be reached to maximize maternal transfer to the egg. Although we do not yet know to what extent pre-egg-laying exposure is essential, we must resolve the question to ensure selection of the most efficient experimental design.

## Treatment of F1 and F2

The goal of limiting the number of eggs taken for incubation and hatching requires careful selection of the cohort of eggs for that purpose. The most critical consideration is to take the eggs during the time of egg production when the full effect of the test substance on the fertility and quality of the eggs and on viability of the young can be manifest. The ovarian cycle in which the yolk is deposited occurs up to 9 days before egg laying (Bacon et al. 1973), and sperm development may occur 17 to 21 days from the earliest stages of spermatogenesis to the time of sperm release (Clulow and Jones 1988; Kirby and Froman 2000; Lin and Jones 1992). In addition, sperm may be stored in the oviduct of the female for as long as 7 to 9 days (Schom and Abbott 1974). For these reasons, it is necessary to expose breeding pairs for a minimum of approximately 6 wk, not taking into consideration the time for the individual parental birds to reach equilibrium from bioaccumulative substances.

In general, a cohort of eggs would be those collected over a 1-wk period. Japanese quail produce 6 to 7 eggs per week during peak production periods, and this number would be the minimum required to provide a sufficient number of eggs per pair to hatch and to allow measures of egg quality and residues. In test groups subjected to reduced egg production and/or fertility, it may also be necessary to consider pooling eggs from an additional cohort.

EDSP is likely to recommend exposing F1 chicks from hatch through egg-laying, and then terminating to provide the maximum opportunity to detect any adverse action associated with the test material. Nevertheless, the potential exists for chicks exposed to the test material to be seriously affected through nonendocrine mechanisms. This effect could result in mortality or other serious effects because the chicks are likely more sensitive than adult birds to a toxicant that would interfere with evaluating potential endocrine-related effects. Rearing the F1 chicks on untreated diet would eliminate this possibility, however, endocrine-related effects associated with this developmental life stage would be missed. We currently lack information to resolve the question as to whether F1 chicks should be exposed or, rather, whether there are endocrine-related effects that

would manifest only from exposure in the juvenile stage. The F2 chicks, in any case, would not be exposed via the diet, and are observed only until sexual differentiation.

## Endpoints

Fitness endpoints assess survival, growth, and reproduction. These endpoints provide information on the general toxicity of a test substance and, to some degree, disruption in endocrine-mediated processes that may affect these conditions. Other physiological endpoints or endocrine-specific endpoints serve as more direct measures of potential disruption of endocrine function.

Important fitness endpoints include toxic signs and health conditions that should be evaluated daily for all birds, during the acclimation, stabilization, and treatment periods. During the treatment period, the parental birds in both the P and F1 generations are observed daily to detect any overt signs of toxicity or other clinical signs. Observations should include mortality and any clinical signs of toxicity such as lethargy, depression, wing droop, ruffled feathers, and lacrimation. Any injuries sustained and subsequent treatment should also be recorded. Clearly moribund individuals or those otherwise in severe distress should be euthanized immediately. Food consumption should be monitored throughout the test, and body weights should be determined at pairing and at the end of the treatment period.

The following egg parameters are monitored from breeding pairs in the P and F1 generations:

- Eggs laid;
- Eggs cracked;
- Eggs broken;
- Description of all egg abnormalities;
- Eggs set;
- Eggshell strength and thickness;
- Fertile/infertile eggs;
- Early viability/embryonic deaths;
- Late viability/embryonic deaths; and
- Egg hatch.

It is suggested that only eggs collected in the eighth week of egg laying are set for incubation to hatch. A small number of eggs will be removed for measures of egg thickness and strength, and any abnormal, cracked, or broken eggs will be recorded and discarded. Eggs that are set and collected before the eighth week will be incubated only until day 8, when fertility and early embryonic development are recorded.

Chicks are also monitored for abnormal conditions at hatch, clinical signs of toxicity, survival, growth and maturation time, and sex ratio. Some measure of neurobehavioral integrity should also be considered for 14-day-old chicks.

Key endocrine endpoints that are included in the test review gross morphology, histopathology, developmental landmarks, and hormone levels. Morphological measures

include gross anomalies, organ/gland weights, and bone lengths (in chicks). Histopathological measures include key organs (thyroid, adrenals, gonads, and brain). In the gonads, attention is given to spermatid count and morphology as direct measures of male competence. Developmental landmarks include feather dimorphism, cloacal gland size, time to sexual maturation, molt timing, and behavior. In addition, steroid and thyroid hormone levels from plasma and fecal/urate samples are measured.

## Data Gaps

Although avian reproduction tests have been standardized, performed, and utilized by regulatory authorities for several decades now, it is still uncertain how this test methodology can be adapted and extended into a two-generation study. The following aspects of a two-generation test method are among the many questionable factors that will require empirically based answers:

- Lack of clear information on source of and metabolic fate of xenobiotics in ovo;
- The effects of antiestrogens in juvenile and sexually mature test species;
- The effects of antiandrogens in the developing embryo or hatchling;
- The effects of thyroid hormone agonists (or thyroid stimulation) on reproduction;
- Interactive effects of endocrine-active substances, especially natural phytosteroids in diets; and
- Reliability of other endpoints (i.e., neurotransmitters, hypothalamic or pituitary hormones).

In addition, the effects of strain differences are not clear to date. Japanese quail strains are generally selected on the basis of high body weight (meat production) or high fecundity (egg production). How this may affect their response to an endocrine-active material is uncertain. Further work is needed to ascertain which qualities are most suitable for toxicity testing in random-bred lines. The optimum statistical approach for evaluating delayed effects should be determined. Finally, improvements are needed in the husbandry and experimental handling of Japanese quail to reduce the occurrence of confounding behaviors and inadvertent mortality. More specifically, information for dealing with factors such as fear, social stress, and injurious pecking would be helpful.

## Summary

The EPA EDSP includes an avian two-generation toxicity test as part of its Tier 2 tests. The test is intended to expose all four critical life stages (in ovo, juvenile, subadults, and adults) to assess any adverse effects associated with a putative endocrine disrupting compound quantitatively. Be-

cause two generations are considered necessary for a test to characterize potential endocrine-related effects adequately, the Japanese quail has been selected as the most suitable test species. Several technical and logistical issues that still require resolution are being addressed through EPA-led projects.

Animal usage is a key concern for this avian test. For this reason, the ultimate method must serve several defined purposes. It will

- Ensure the minimum number and most efficient use of birds;
- Demonstrate the added value of a two-generation test method, replacing the existing one-generation avian reproduction test method; and
- Link the avian two-generation test method used in the EDSP to existing avian testing frameworks to support pesticide and industrial chemical regulatory programs.

## References

- ASTM [American Society for Testing and Materials]. 1990. Standard Practice for Conducting Reproductive Studies with Avian Species. Vol 11.94. Philadelphia: ASTM. p E1062-E1086.
- Bacon WL, Nestor KE, Renner PA. 1973. Ovarian follicular development in egg and growth lines of Japanese quail. *Poultry Sci* 52:1195-1199.
- Bennett JK, Bennett RS. 1990. Effects of dietary methyl parathion on northern bobwhite egg production and eggshell quality. *Environ Toxicol Chem* 9:1481-1485.
- Bennett RS, Bentley R, Shiroyama T, Bennett JK. 1990. Effects of the duration and timing of dietary methyl parathion exposure on bobwhite reproduction. *Environ Toxicol Chem* 9:1473-1480.
- Bennett RS, Brugger K, Fairbrother A, Leopold A, Mastrota N, and Ottinger MA. 2001. Discussion Document of Prevalidation of an Avian Two-Generation Toxicity Test with the Japanese Quail. Draft Document March 2001. Paris: Organisation for Economic Cooperation and Development.
- Bennett RS, Ganio LM. 1991. Overview of Methods for Evaluating Effects of Pesticides on Reproduction in Birds. (Publication 600/3-91/048.) Corvallis OR: US Environmental Protection Agency.
- Bitman J, Cecil HC. 1970. Estrogenic activity of DDT analogs and polychlorinated biphenyls. *J Agric Food Chem* 18:1108-1112.
- Bitman J, Cecil HC, Harris SJ, Fries GF. 1968. Oestrogenic activity of o,p-DDT in the mammalian uterus and avian oviduct. *Science* 162:371-372.
- Carson R. 1962. *Silent Spring*. Boston: Boston University Press.
- Clulow J, Jones RC. 1988. Studies of fluid and spermatozoal transport in the extratesticular genital ducts of the Japanese quail. *J Anat* 157:1-11.
- Colborn T, Clement C, eds. 1992. *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton: Princeton Scientific Publishing.
- Colborn T, Dumanoski D, Meyers JP. 1996. *Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival? A Scientific Detective Story*. New York: Plume/Penguin Books.
- Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101:378-384.
- Collins BT. 1994. *The Avian Reproduction Study: Distribution of the Control Data and Statistical Power Analyses*. (Technical Report Series no. 214.) Toronto Ontario Canada: Canadian Wildlife Service.
- Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, Hoffman JL, Morrow MS, Rodier DJ, Schaeffer JE, Touart LW, Zeman MG, Patel YM. 1998. Environmental endocrine disruption: An effects assessment and analysis. *Environ Health Perspect* 106:11-56.
- Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G. 2002. *Global Assessment of the State-of-the-Science of Endocrine Disruptors*. International Programme on Chemical Safety. WHO/PCS/EDC/02.2. Geneva: World Health Organization.
- Dell RB, Holleran S, Ramakrishnan R. 2001. Sample size determination. *ILAR J* 43:207-213.
- EDSTAC [Endocrine Disruptor Screening and Testing Advisory Committee]. 1998. Final Report. Available at <http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm>.
- EPA [Environmental Protection Agency]. 1982. *Pesticide Assessment Guidelines*. Subdivision E. Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024. Washington DC: US Environmental Protection Agency.
- EPA [Environmental Protection Agency]. 1985. *Toxic Substances Control Act (TSCA)—Test Guidelines for Reproduction and Fertility Effects*. Federal Register 50(188):39426-39436.
- Federal Register. 1998a. *Endocrine Disruptor Screening Program* 63:42852 (August 11, 1998).
- Federal Register. 1998b. *Endocrine Disruptor Screening Program: Statement of Policy; Notice* 63:71542 (December 28, 1998).
- FQPA [Food Quality Protection Act]. 1996. Washington DC: GPO.
- Gray LE Jr, Kelce WR, Wiese T, Tyl R, Gaido K, Cook J, Klinefelter G, Deauliniers D, Wilson E, Zacharewski T, Waller C, Faoster P, Laskey J, Reel J, Geisy J, Laws S, McLachlan J, Breslin W, Cooper R, DiGiulio R, Johnson R, Purdy R, Mihaich E, Safe S, Sonneshchein C, Welshons W, Miller R, McMaster S, Colborn T. 1997. Endocrine screening methods workshop report: Detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. *Reprod Toxicol* 11:719-750.
- Hertz R. 1985. The estrogen problem. Retrospect and prospect. In: McLachlan JA, ed. *Estrogens in the Environment. II: Influences on Development*. New York: Elsevier North Holland. p 1-11.
- Hutchinson T, Brown R, Brugger K, Campbell P, Holt M, Lange R, McCahon P, Tattersfield L, van Egmond R. 2000. Ecological risk assessment of endocrine disruptors. *Environ Health Perspect* 108:1007-1013.
- ICCVAM [Interagency Coordinating Committee for the Validation of Alternative Methods]. 2000. *ICCVAM Authorization Act, PL 106-545*. Research Triangle Park: NIEHS.
- Kirby JD, Froman DP. 2000. Reproduction in male birds. In: Whittow GC, ed. *Sturkie's Avian Physiology*. San Diego: Academic Press. p 597-615.
- Lin M, Jones RC. 1992. Renewal and proliferation of spermatogonia during spermatogenesis in the Japanese quail, *Coturnix coturnix japonica*. *Cell Tiss Res* 267:591-601.
- McLachlan JA, ed. 1980. *Estrogens in the Environment*. Amsterdam: Elsevier.
- Mineau P, Boersma DC, Collins B. 1994. An analysis of avian reproduction studies submitted for pesticide registration. *Ecotoxicol Environ Safety* 29:304-329.
- Nelson JA, Struck RF, James R. 1978. Estrogenic activities of chlorinated hydrocarbons. *J Toxicol Environ Health* 4:325-339.
- NRC [National Research Council]. 1999. *Hormonally Active Agents in the Environment*. Washington DC: National Academy Press.
- OECD [Organisation for Economic Cooperation and Development]. 1993. *OECD Guidelines for the Testing of Chemicals. Section 2—Effect on Biotic Systems: Test Guideline 206: Avian Reproduction Test (adopted April 1984)*. Paris: OECD.
- Ottinger MA, Brinkley HJ. 1978. Testosterone and sex-related behavior and morphology: Relationship during maturation and in the adult Japanese quail. *Hormones Behav* 11:175-182.
- Ottinger MA, Brinkley HJ. 1979a. Testosterone and sex-related physical characteristics during the maturation of the male Japanese quail (*Coturnix coturnix japonica*). *Biol Reprod* 20:905-909.
- Ottinger MA, Brinkley HJ. 1979b. The ontogeny of crowing and copulatory behaviour in Japanese quail. *Behav Process* 4:43-51.

- Ottinger MA, vom Saal FS. 2002. Impact of environmental endocrine disruptors on sexual differentiation in birds and mammals. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, eds. *Hormones, Brain and Behavior*. Vol 4. New York: Elsevier Science and Technology Books. p 325-383.
- Rattner BA, Sileo L, Scanes CG. 1982. Hormonal responses and tolerance to cold of female quail following parathion ingestion. *Pest Biochem Physiol* 18:132-138.
- Scanes CG, McNabb FMA. 2003. Avian models for research in toxicology and endocrine disruption. *Avian Poultry Biol Rev* 14:21-52.
- Schom CB, Abbott UK. 1974. Studies with bobwhite quail: Reproductive characteristics. *Poultry Sci* 53:1860-1865.
- Springer TA, Collins BT. 1999. Statistical power of tests in the avian reproduction study: A summary and interpretation of available information. Report presented May 23, 1999, to the OECD Expert Group on Avian Reproductive Toxicity Testing in Leipzig, Germany.
- Stromborg KL. 1981. Reproductive tests of diazinon on bobwhite quail. In: Lamb DW, Kenaga EE, eds. *Avian and Mammalian Wildlife Toxicology: Second Conference*. ASTM STP 757. Philadelphia: ASTM. p 19-30.
- Stromborg KL. 1986. Reproduction of bobwhites fed different dietary concentrations of an organophosphate insecticide, methamidophos. *Arch Environ Contam Toxicol* 15:143-147.
- Yoshimura Y, Tamura Y, Nishkori M, Okamoto T. 2000. Effects of diethylstilberol intake during growing phase on the reproductive organs in Japanese quail (*Coturnix japonica*). *Jpn Poultry Sci* 37:323-333.