Field Deployable Methods for Monitoring Endocrine Effects in Birds

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The goal of this study is to continue the development and field verification of research methods for monitoring avian exposure to endocrine active substances and to quantify reproductive effects in wild bird populations. The study objectives will be accomplished in two phases. Phase One objectives are: (1) to capture and expose California quail (*Callipepla californica*) to exogenous estrogen via implanted estrogen pumps during egg development and monitor levels of excreted estrogen and testosterone of exposed birds and control birds until nest initiation; (2) to monitor nest success through the breeding season until egg hatch; (3) to capture the hatchlings [first filial (F1) generation] during the first 10 days post-hatch, to determine the sex of each hatchling, to permanently mark them for later identification, and to release them to be recaptured in Phase Two. The Phase Two objectives are: (1) to recapture surviving F1 birds in early Spring 2004 prior to nesting and equip them with radio transmitters; (2) to radio-tag and monitor F1 birds through the 2004 nesting season using radio-telemetry and monitor nesting success to quantify the reproductive behavior and success of the F1 generation (from both treated and control P1 parents) in the absence of additional exposure (no exposure beyond *in ovo*); and (3) to capture hatchlings (F2 generation) of monitored F1 adults in spring and summer of 2004 to determine sex ratio.

Repetitive blood sampling is a logistically difficult method of monitoring chemical-induced modulations of circulating steroid hormones in free-ranging birds. We experimented with developing field-deployable methods for monitoring temporal modulations in excreted hormone levels caused by endocrine disrupting chemicals. Early experimentation involved subcutaneous implantation of time-released 17-β estradiol capsules (Innovative Research of America, Sarasota, Florida) and serial collection of fecal-urate droppings from captive wild house finches (*Callipepla californica*) and radio-tagged, free-ranging California quail (*Carpodacus mexicanus*). Results of the fecal-urate estrogen assay for the captive, estrogenized, female house finches and estrogenized free-ranging female California quail indicated that circulating estrogen levels rose very rapidly and then declined relatively quickly after implantation of the constant-release estrogen pumps. These observations differed from expected results. We had anticipated that measured estrogen levels would rise rapidly and remain at a near-constant level until the exogenous estrogen implants exhausted its estrogen supply and then measured estrogen levels would decline to pre-implant levels rapidly.

Three possible hypotheses were formulated to explain possible reasons for the observed pattern of apparent decline in circulating estrogen following implantation of birds with time-release estrogen capsules proven previously to release estrogen at a consistent rate in mammals: (1) the experimental species have a feed-back mechanism triggered by exogenous estrogen that allows for rapid estrogen metabolism or elimination; (2) the time-release estrogen capsules failed to release their estrogen content in equal daily amounts; and (3) the implanted time-release estrogen capsules were quickly encased in scar tissue, rendering them ineffective. Correct interpretation of the results of experimentation involving the estrogen pumps hinged on understanding the mechanism controlling the circulating estrogen patterns observed. The hypothesis that the constant-release, 17β estradiol capsules may have failed to release consistent daily doses of estradiol was rejected based on the temporal pattern of excreted estrogen observed in group 2 following their implanted with the capsules, which were removed from treatment group 1. The hypothesis that the capsule may be rendered ineffective due to encasement by scar tissue was also ruled out. The results of this study imply that house finches, and possibly other avian species, have a mechanism that controls the influence of exogenous estrogen on circulating estrogen levels. One possible mechanism for this control is that a sudden increase in circulating estrogen stimulates production of a hepatic enzyme that metabolizes estrogen until circulating levels return to normal. Other mechanisms are also possible.

Implications: The results of this project explain why, in previous research, we detected no change in reproductive performance in California quail or house finches when exposed to exogenous estrogen via the described estrogen pumps. More importantly, the results suggest that some species of birds may be far less sensitive to exogenous estrogen than proposed by regulators and others. This may have important implications for future ecological risk assessments with regard to the effects of estrogenic compounds in the environment on avian health and reproduction.

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Presentations:

Brewer, L.W., Fairbrother, A., McQuillen, H.L., Hunt, K.E., and Tank. S.L. (2003). Evaluating exposure and reproductive effects of endocrine active substances in wild birds. Platform presentation (invited paper) at the 2003 Annual Meeting of The Society of Environmental Toxicology and Chemistry, Austin, TX.

Brewer, L.W., Fairbrother, A., McQuillen, H.L., Hunt, K.E., and Tank. S.L. (2003). Evaluating exposure and reproductive effects of endocrine active substances in wild birds. Platform presentation at the American Chemistry Council 2003 Long Range Research Initiative Annual Science Meeting, Herndon, VA.

Brewer, L.W., Tank, S., Murray, N., and Hunt, K. (2004). Development and verification of field-deployable methods for evaluating exposure and effects of endocrine active substances in wild bird populations. Poster presented at 2004 ACC Long Range Research Initiative Science Meeting, Miami, FL.

Peer-reviewed publications: None to date.

Other publications: None to date.

Other references listed:

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