TOXICITY AND REPRODUCTIVE EFFECTS
OF 2,3,7,8- TETRACHLORODIBENZO- p -DIOXIN
IN RING-NECKED PHEASANT HENS

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Hen pheasants (Phasianus colchicus) injected with graded single doses of TCDD (6.25,
25, or 100 µg/kg) exhibited delayed-onset body weight loss and mortality—classic
signs of the wasting syndrome. The lowest single dose of TCDD to produce this effect
was 25 µg/kg. When hen pheasants were treated weekly with far lower doses of TCDD
(0.01-1.0 µg/kg/wk) for 10 wk, signs of the wasting syndrome and mortality were also
produced. The lowest cumulative TCDD dose required to produce the response, using
a weekly dosing regimen, was 16 µg/kg. Furthermore, using this dosing regimen,
egg production by hens treated with a cumulative TCDD dose of 10 µg/kg was re-
duced, as was hatchability of their eggs. We conclude that hen pheasants are respon-
sive to the overt toxic effects of TCDD and that the lowest cumulative dose of TCDD
that produces overt signs of toxicity, 10 µg/kg, also reduces egg production and egg
hatchability.

INTRODUCTION

Chlorinated dibenzo-p-dioxins, particularly the 2,3,7,8-chlorine-substituted congener (TCDD), can produce a variety of toxic responses in
laboratory animals (McConnell, 1980; Morrissey and Schwetz, 1989), but
less is known about its effects on wildlife. From a population ecology

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perspective, the most important effects are those that impact upon reproductive success. A population that fails to replace its members through reproduction will eventually be lost. Adult male or female birds living in a TCDD-contaminated environment might accumulate, for example, a body burden of TCDD that is sufficiently high to cause infertility. Alternatively, a percentage of the female TCDD body burden might be translocated to the egg and cause embryo mortality. Either type of effect would be detrimental to the reproductive success of the species. Injection of graded doses of TCDD or coplanar PCB congeners (that act by the same Ah receptor-mediated mechanism) into fertilized eggs of various bird species (chicken (Gallus domesticus), pheasant (Phasianus colchicus), bluebird (Sialia sialis), goose (Anser anser), herring gull (Larus argentatus), mallard (Anas platyrhynchos), goldeneye (Bucephala clangula), and black-headed gull (Larus ridibundus)) produce embryo mortality (Allred and Strange, 1981; Brunstrom, 1988; Brunstrom and Reutergardh, 1986; Martin et al., 1989; Nosek et al., 1991). However, embryotoxic effects of halogenated aromatic hydrocarbons are not the only way in which reproductive performance of wild bird species can be impaired. Diminished parental attentiveness at the nest has also been attributed to organochlorine contamination of certain wild bird species (Kubiak et al., 1989; Peakall et al., 1978). The body condition of female birds exposed to halogenated aromatic hydrocarbons can be too poor to support the increased physiological demands of egg laying, resulting in decreased egg production (Dahlgren and Linder, 1971; Platonow and Reinhart, 1973; Scott et al., 1975). Also, eggshell thinning has occurred in a number of species exposed to organochlorine contamination (Hickey and Anderson, 1968). Although halogenated aromatic hydrocarbon exposure can adversely affect reproductive function of adult birds, the developing bird embryo is the most sensitive life stage. This is illustrated by increases in bird embryo mortality occurring at PCB exposure levels that did not reduce fertility of the adult bird (Keplinger et al., 1971; Lillie et al., 1974; Tumasonis et al., 1973).

The objective of the present study was to assess the effects of TCDD exposure on ring-necked pheasant hens. Mortality and reductions in body weight caused by a single dose of TCDD as well as by 10 weekly doses of TCDD were examined. In addition, reproductive capacity of TCDD-treated hens was evaluated by measuring total egg production and hatchability of eggs.

**METHODS**

**Animals and Chemicals**

Sexually mature (40-45 wk old) hen pheasants (Phasianus colchicus; 0.9-1.3 kg) obtained from the Wisconsin Department of Natural Re-
sources Game Farm (Poynette, Wis.) were housed in galvanized steel cages (one bird/cage) in a room kept at 18 ± 2°C and ambient humidity. Commercial pheasant breeder ration (3.5% fat, 22% protein; Carver’s Feed and Supply Co., Madison, Wis.) and water were provided ad libitum. After adapting to the housing and feeding conditions for 2 wk, the hen pheasants were treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 98% purity; Cambridge Isotope Laboratories, Woburn, Mass.) or vehicle (corn oil:acetone, 19:1, v:v).

**Acute Toxicity**

Hens (n = 4/dose) were given single graded doses of TCDD (6.25, 25, or 100 µg/kg) or an equivalent volume of vehicle, ip (1.0 ml/kg). Injections were favored over oral dosing for the administration of known quantities of TCDD. Hens were observed daily for mortality or other signs of toxicity and were weighed weekly. A photoperiod of 8 h light:16 h dark was maintained throughout the experiment. All hens alive 11 wk after treatment were sacrificed by carbon dioxide asphyxiation.

**Subchronic Toxicity**

Once a week for 10 wk, hens were weighed and given by ip injection either a graded dose of TCDD (0.01, 0.1, or 1.0 µg/kg; 7 hens/dose) or vehicle (1.0 ml/kg; 4 hens). For the first 8 wk after the start of TCDD or vehicle treatment, the hens were maintained on an 8 h light:16 h dark photoperiod. Thereafter, the photoperiod was changed to 14 h light:10 h dark to bring the hens into egg production. The final two weekly injections were administered during the latter photoperiod. One week after changing the photoperiod hens were first bred to roosters. Six roosters were used for breeding. Once each week two hens were placed with one rooster in a large cage for a period of 48 h, which allowed for copulation to occur a number of times for each hen. Pairings of hens to roosters were alternated each week. Egg production began during wk 11. Eggs were collected daily and the first 15 eggs from each hen were placed into a Jamesway incubator (model 252; 38°C, 85% humidity) and incubated to hatching, or until it could be positively determined that the developing embryo was dead or the egg was inviable. At this time the egg was removed from the incubator and stored at −20°C. Successful hatching was defined as a chick emerging completely from the shell alive. Egg collection continued until each hen in the control group laid 30 eggs, at which time all hens in the control and 0.01 and 0.1 µg TCDD/kg/wk dose groups were euthanized. Due to an observed delay in onset of egg production, hens in the 1.0 µg TCDD/kg/wk dose group were kept in egg production for an additional 5 wk, then euthanized.
Statistical Analysis

One-way analysis of variance, followed by least significant difference (LSD) tests, was used to determine dose-related differences between treatment groups in body weight, adult mortality, egg production, and embryo mortality of eggs from TCDD-treated hens. Data were not transformed. A significance level of $p < 0.05$ was used. Embryo mortality was corrected for observed control group mortality according to Abbott's formula (Hewlett and Plackett, 1979). Linear regression of cumulative dose versus embryo mortality was then used to estimate a 50% increase in embryo mortality over controls. Eggshell thickness index for eggs collected in the subchronic treatment experiment ($n = 10-34$) was calculated by the method of Ratcliffe (1970).

RESULTS

Acute Toxicity

Hen pheasants treated with a graded single dose of TCDD showed a dose-dependent increase in cumulative percent mortality (Fig. 1). All hens receiving 100 μg TCDD/kg died within 6 wk of treatment. No mor-
Mortality was observed in hens treated with vehicle (control) or 6.25 µg TCDD/kg. Mortality in the 25 µg TCDD/kg group was intermediate between the control and 100 µg TCDD/kg group. In the two dose groups in which mortality was observed there were time-mortality differences. Onset of mortality in hens treated with 25 µg TCDD/kg occurred after a longer delay period than in hens given 100 µg TCDD/kg. Anorexia and cachexia preceded mortality in all cases. Daily feed intake was estimated by monitoring the change in feed level in individual food hoppers. In control hens, one third to the two thirds of the feed in each hopper was eaten or scattered each day. No feed was consumed or scattered from the hoppers by hens eliciting signs of toxicity.

Mortality in TCDD-treated hen pheasants was preceded by a dose-dependent decrease in body weight (Fig. 2). Control hens maintained body weight during the 11-wk duration of the study, whereas hens treated with the lowest dose of TCDD, 6.25 µg/kg, lost about 5% of their body weight. In contrast, hens treated with the two lethal doses of TCDD, 25 or 100 µg/kg, lost 25-40% of their body weight. The magnitude

![Graph showing the effect of a single dose of TCDD on the percent of initial body weight. Hen pheasants were treated on wk 0 with a single dose of either TCDD (6.25, 25, or 100 µg/kg) or vehicle (0 µg/kg). Initial body weight (mean ± SE, n = 4/group) of the pheasants used in the 0, 6.25, 25, and 100 µg/kg dose groups was 1092 ± 106, 1254 ± 210, 1128 ± 183, and 1225 ± 131 g, respectively, and each value was set equal to 100%. The asterisk indicates a significant difference from control (p < .05).]
of weight loss was greatest in hens treated with the highest dose of TCDD.

Subchronic Toxicity

No mortality occurred in hen pheasants treated with 10 weekly injections of vehicle (control), 0.01, or 0.1 μg/kg of TCDD (cumulative TCDD doses of 0, 0.1, and 1.0 μg/kg, respectively). On the other hand, in hen pheasants treated with 1.0 μg/kg TCDD for 10 wk (cumulative TCDD dose 10 μg/kg), delayed onset of mortality occurred in 57% of the birds (Fig. 3).

Body weight increased in all TCDD groups during the 10-wk treatment period, but the magnitude of the increase in body weight was less for hen pheasants treated with the highest cumulative dose of TCDD, 10 μg/kg (Fig. 4). During the initial stages of egg production (wk 10–13) body weight was either maintained or decreased in all groups, including control. However, during the later stages of egg production only hens treated with the highest cumulative dose of TCDD, 10 μg/kg, showed a

![CUMULATIVE TCDD DOSE](image)

**FIGURE 3.** Effect of a cumulative dose of TCDD on mortality. Hen pheasants were treated weekly for 10 wk with TCDD (0.01, 0.1, or 1.0 μg/kg/wk; cumulative dose = 0.1, 1.0, or 10 μg/kg) or vehicle (0 μg/kg/wk; cumulative dose = 0 μg/kg). There were seven pheasants per dose of TCDD and four pheasants per dose of vehicle. The asterisk indicates a significant difference from controls (p < .05).
steady decline in body weight. All other treatment groups maintained body weight.

Egg production in hen pheasants treated with vehicle or the two lowest cumulative doses of TCDD (0.1 or 1.0 μg/kg) was similar (Fig. 5). However, cumulative egg production in pheasant hens treated with the highest cumulative dose of TCDD (10 μg/kg) was less than control \(p < .05\). In this high TCDD dose group there was a wide range of egg production. One hen, which had produced no eggs, was sacrificed due to poor condition at the end of wk 16. In the remaining six hens alive through wk 17, egg production ranged from 0 to 32 eggs/hen. Eggshell thickness index was not different between control and TCDD-treated hen pheasants.

The cumulative TCDD dose-response relationship for effects on hen mortality, hen body weight, egg production, and embryo mortality in fertilized eggs of TCDD-treated hen pheasants is shown in Figure 6. A cumulative TCDD dose to the hen of 10 μg/kg not only caused overt toxicity in the hen, as evidenced by increased mortality (Fig. 6a), decreased body weight (Fig. 6b), and decreased egg production (Fig. 6c), it...
also caused a significant increase in cumulative percent mortality of the embryos from the fertilized eggs of these hens (Fig. 6a). Mortality occurred very early in development, usually within 72 h, as evidenced by lack of discernible embryos in the unhatched eggs. Egg fertility did not appear to be adversely affected, because control group embryo mortality was close to that experienced in commercial pheasant hatching operations (Don Bates, Wisconsin Department of Natural Resources, personal communication, 1990). The cumulative TCDD dose to the hen that produced a 50% increase (above control) in embryo mortality was estimated to be 4.5 μg/kg.

DISCUSSION

Overt Toxicity

Reproduction in ring-necked pheasant hens was impaired at cumulative TCDD doses that caused overt toxicity. Other investigators have
TCDD TOXICITY IN HEN PHEASANTS

FIGURE 6. Effect of a cumulative dose of TCDD on hen mortality, body weight loss, egg production, and embryo mortality. Hen pheasants were treated weekly for 10 wk with TCDD (0.01, 0.1, or 1.0 µg/kg/wk; cumulative dose = 0.1, 1.0, or 10 µg/kg) or vehicle (0 µg/kg/wk; cumulative dose = 0 µg/kg). There were seven pheasants per dose of TCDD and four pheasants per dose of vehicle. Values are mean ± SE. Panel A: hen mortality at wk 17 (diamond symbol is mortality at wk 23 for 10 µg/kg group). Panels B and C: percent of initial body weight and total egg production per hen at wk 16. Panel D: percentage embryo mortality (corrected for control mortality) for fertilized eggs from TCDD-treated or vehicle-treated hens. The asterisk indicates a significant difference from control (p < .05).

shown similar effects of TCDD in other bird species. Grieg et al. (1973) found that chickens given a single oral dose of TCDD (25–50 µg/kg) died within 12–21 d following treatment. Prior to death the chickens lost body weight and were in poor condition. These results are similar to those of the present study where a single ip dose of TCDD (25–100 µg/kg) caused delayed-onset body weight loss and mortality. Furthermore, we found that when TCDD was administered weekly for 10 wk to hen pheasants, the wasting syndrome that precedes mortality (Christian et al., 1986; Seefeld et al., 1984) could be produced by a cumulative TCDD dose as low as 10 µg/kg. We conclude that hen pheasants are responsive to the overt toxic effects of TCDD.

Egg Production

A number of halogenated aromatic hydrocarbons have been shown to decrease egg production in birds. Dahlgren and Linder (1971) fed hen
pheasants 50 mg of polychlorinated biphenyls (PCBs) in capsules weekly for 17 wk and found that egg production was reduced. Platonow and Reinhart (1973), Scott et al. (1975), and Lillie et al. (1974) found that PCB exposure decreases egg production in chickens, while Polin and Ringer (1978) demonstrated the same effect from polybrominated biphenyls (PBBs). Our finding that a cumulative dose of TCDD that causes overt toxicity in hen pheasants also decreases egg production in this species is, therefore, consistent with the known effects of halogenated aromatic hydrocarbons in such sensitive bird species as the chicken and pheasant. Other bird species, namely, mallards and screech owls (Otus asio), are more resistant to PCB overt toxicity and reproductive effects (Custer and Heinz, 1980; McLane and Hughes, 1980).

Embryo Mortality and Fertility

Dahlgren and Linder (1971) and Dahlgren et al. (1972) found that PCB exposure of hen pheasants adversely affected the viability of embryos at the time of hatching. Kepplinger et al. (1971) found poor hatchability in chicken eggs from hens fed 10 or 100 ppm Aroclor 1242 or 100 ppm Aroclor 1254. Tumasonis et al. (1973) treated chicken hens with 50 ppm Aroclor 1254 in water for 6 wk and found that egg fertility was not affected, but egg hatchability dropped to near zero after wk 3 of treatment and did not return to normal until wk 17 after PCB exposure was stopped. Platonow and Reinhart (1973) found that hatchability of eggs from chickens receiving 50 ppm PCB was significantly reduced when no pronounced differences in fertility were observed. Lillie et al. (1974) found that fertility of white leghorn hens fed 20 ppm PCB in the diet was normal while the hatchability of their eggs was reduced. Scott et al. (1975) found that 10–20 ppm dietary PCB reduced hatchability of chicken eggs, and Polin and Ringer (1978) found decreased hatchability of eggs from chickens fed 45 ppm PBB in the diet. In light of these various findings, it was predictable that exposing hen pheasants to a high enough body burden of TCDD would reduce hatchability of the fertilized eggs laid by these birds. In the present study, the lowest cumulative TCDD dose in the hen pheasant that produced this effect was 10 μg/kg.

Relevance to Field Observations

The present study shows that overt toxicity and reproductive effects occur in hen pheasants exposed to TCDD in the laboratory. Reproductive and developmental toxicity have also been observed in field studies. Forster’s tern (Sterna forsteri) eggs from Green Bay, Lake Michigan, with mean TCDD residue levels of 37 ppt, showed decreased hatching success when compared to eggs from an inland “clean” colony at Lake Poygan, with mean TCDD residue levels of 8 ppt (Hoffman et al., 1987). Great blue heron (Ardea herodius) eggs collected from colonies in British Columbia, Canada, contained TCDD residues as high as 211 ppt, but did
not show a TCDD-related increase in embryo mortality. However, a depression of growth and the presence of edema were observed in heron chicks, suggestive that dioxins at the levels found in the environment had an adverse effect on development (Hart et al., 1991).

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