APPENDIX A

ATSDR MINIMAL RISK LEVEL AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical Name: DDT
CAS Number: 50-29-3
Date: September 2002
Profile Status: Third Draft Post Public
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 47m
Species: Mice

Minimal Risk Level: 0.0005 [X] mg/kg/day [ ] ppm

Reference: Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996; Talts et al. 1998.

Experimental design and effects noted: The acute oral MRL is based on results from a group of studies conducted by the same group of investigators in which the most significant finding was the presence of altered motor behavior in adult mice treated with DDT perinatally. Groups of 10-day-old male NMRI mice were treated by gavage with a single dose of 0 (vehicle control) or 0.5 mg DDT/kg in a fat emulsion vehicle by gavage (Eriksson et al. 1990a). At the age of 4 months, the mice were subjected to behavioral tests of spontaneous activity (locomotion, rearing, and total activity). Tests were conducted for 1 hour, and scores were summed for three 20-minute periods. During the last 40 minutes of testing, the treated mice showed significantly more activity than untreated controls. This was interpreted as disruption of a simple, non-associative learning process, (i.e., habituation), or a retardation in adjustment to a new environment. These same results were reported in a later paper (Eriksson et al. 1990b) in which the authors also reported results of neurochemical evaluations conducted 2–3 weeks after behavioral testing. They measured muscarinic acetylcholine (MACh) receptor density and choline acetyltransferase (ChAT) activity in the cerebral cortex and hippocampus (MACh also in striatum), and also measured K^+-stimulated ACh release from cerebral cortex slices. In addition, five 10-day-old mice were administered 0.5 mg ^{14}C-DDT and the radioactivity in the brain was assayed 24 hours, 7 days, or 1 month after dosing. The results showed that K^+-evoked ACh release in treated mice was significantly increased relative to controls, ChAT activity was not changed in the cerebral cortex or hippocampus, and the density of MACh was not significantly changed in the hippocampus or striatum, but a decreasing trend was seen in the cerebral cortex. DDT-derived radioactivity could be detected until day 7 after dosing, but none could be detected 1 month after dosing.

Previous studies have shown a significant increase in density of MACh in the cerebral cortex of 10-day-old mice 7 days after dosing, but not at 1 day post-exposure compared to controls (Eriksson and Nordberg 1986). No increased binding was noted in the hippocampus either 1 or 7 days post-treatment. This was further investigated by evaluating the proportion of high- and low-affinity binding sites and the affinity constants of the muscarinic receptors. A significant increase in the percentage of low-affinity binding sites accompanied by a significant decrease in high-affinity binding sites was measured in the cerebral cortex 7 days post-exposure. No significant changes in affinity constants were noted. According to the authors, these low-affinity binding sites correspond to the M_1 receptor in the cerebral cortex, which are thought to be associated with neuronal excitation. No changes were observed in the sodium-dependent choline uptake system in the cerebral cortex 7 days post-exposure.

In a follow-up study, Eriksson et al. (1992) treated 3-, 10-, and 19-day-old mice, and conducted behavioral testing and neurochemical evaluations at 4 months of age. As previously published, mice

treated at 10 days exhibited hyperactivity relative to controls and also a significant decrease in the density of MACh in the cerebral cortex. No such changes were seen in mice treated at 3 or 19 days old. The authors suggested that the changes in MACh density and behavior might be the consequence of early interference with muscarinic cholinergic transmission specifically around the age of 10 days. In subsequent studies by the same group, 5- and 7-month-old mice were tested (Eriksson et al. 1993; Johansson et al. 1995). At both time points, mice treated with DDT perinatally showed increased spontaneous motor activity relative to controls, and decreased density of MACh in the cerebral cortex. No changes were seen regarding percentages of high- or low-affinity muscarinic binding sites in the cerebral cortex. Mice in these studies were also treated orally at the age of 5 months with the type I pyrethroid insecticide, bioallethrin, and tested for motor activity at this age (Eriksson et al. 1993) and at 7 months (Johansson et al. 1995). In general, mice treated with DDT at the age of 10 days and later with bioallethrin showed increased motor behavior relative to those treated with bioallethrin alone, suggesting a DDT-induced increased susceptibility to bioallethrin. Mice treated first with DDT and later on with bioallethrin also showed increased difficulties in learning a skill, such as the swim maze test, compared with untreated mice, mice treated with DDT alone, or mice treated with bioallethrin alone (Johansson et al. 1995). Also, treatment with DDT followed by bioallethrin significantly increased the density of muscarinic receptors in the cerebral cortex relative to DDT alone. This increase was later attributed to increased expression of muscarinic receptor m4 mRNA (Talts et al. 1998). In yet another study from this group, paraoxon replaced bioallethrin, and the mice were tested at 5 and 7 months (Johansson et al. 1996). In addition, acetylcholinesterase activity was measured in cerebral cortex of 5-month-old mice and MACh and nicotinic cholinergic receptors in cortex of 7-month-old mice. Relevant new findings include that: (1) DDT did not significantly alter acetylcholinesterase activity; (2) DDT did not alter the effects of paraoxon on acetylcholinesterase activity (decreased); (3) DDT altered (increased or decreased) some of motor responses due to paraoxon alone at 7 months but not at 5 months; (4) none of the treatments altered performance in the swim maze test; and (5) none of the treatments altered the density of nicotinic cholinergic receptors in the cortex.

In this series of studies, two responses seem to be consistent from study to study in mice treated with DDT perinatally and tested as adults, a decrease in the density of muscarinic cholinergic receptors in the cerebral cortex and increased spontaneous motor activity. It is not clear whether there is a causality relationship. DDT also altered some motor responses induced by other pesticides, but a pattern was not always clear. The investigators interpreted the latter findings as DDT inducing changes early in the brain that translated into increased susceptibility to other pesticides later in life. The DDT-induced increase in spontaneous motor activity at the dose of 0.5 mg/kg is considered a less serious LOAEL.

Dose and end point used for MRL derivation: 0.5 mg/kg; neurodevelopmental effects.

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

The DDT in this experiment was administered in a fat emulsion vehicle via gavage. There was no adjustment made for effects of the fat vehicle on absorption because neonatal children are likely to be exposed to DDT via high fat breast milk.

Was a conversion factor used from ppm in food or water to a mg/body weight dose?
No

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: NA

Was a conversion used from intermittent to continuous exposure?
No

Agency Contact (Chemical Manager): Obaid Faroon

APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical Name: DDT
CAS Number: 50-29-3
Date: September 2002
Profile Status: Third Draft Post Public
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 60r
Species: Rat

Minimal Risk Level: 0.0005 [X] mg/kg/day [ ] ppm
In this dietary study the amount of DDT added to the food was measured, but the actual food consumption and body weights of the rats were not measured. The calculated value for the NOAEL on which the MRL is based ranges from 0.05 to 0.09 mg/kg/day depending on the food consumption values used to calculate the actual dose of DDT consumed. More details are provided below in the discussion of conversion factors.

In protecting public health ATSDR recommends using the conservative lower end of this calculated NOAEL range, 0.05 mg/kg/day, to derive an intermediate-duration MRL of 0.0005 mg/kg/day. This MRL value is consistent with and supported by the acute-duration MRL of 0.0005 mg/kg/day, particularly if intermediate exposure were to occur during the sensitive critical window of development (postnatal day 10 in the mouse model) identified in the acute duration MRL exposure studies (Eriksson and Nordberg 1986; Eriksson et al. 1990a, 1990b, 1992, 1993; Johansson et al. 1995, 1996).

Reference: Fitzhugh and Nelson 1947; Laug et al. 1950

Experimental design: Groups of male and female Osborne-Mendel rats (15/sex/group) were administered technical DDT(dissolved in corn oil) added to the diet at dosage levels of 0, 1, 5, 10, or 50 ppm for 15–27 weeks. This study was essentially designed to examine whether DDT accumulates in adipose tissue and to what extent, how age and dose level affect accumulation, and how rapidly it is eliminated. Seventy-seven rats were used for microscopic evaluation of only the liver and kidney. This was based on findings from a previous study from the same group (Fitzhugh and Nelson 1947, see below) in which higher dietary levels of DDT had been used. Based on the previous findings, only the liver was expected to show microscopic changes. Although not explicitly stated, it is assumed that morphologic evaluations were conducted at the times when DDT levels in fat were determined (after 15, 19, 23, and 27 weeks of treatment).

Effects noted in study and corresponding doses: These dose ranges were calculated as shown below in the discussion of conversion factors. There were no morphologic alterations in the kidneys. Liver alterations were noticed at the 5 ppm (0.25–0.5 mg/kg/day) dietary level of DDT and higher, but not at 1 ppm (0.05–0.09 mg/kg/day). Liver changes consisted of hepatic cell enlargement, especially in central lobules, increased cytoplasmic oxyphilia with sometimes a semihyaline appearance, and more peripheral location of the basophilic cytoplasmic granules. Necrosis was not observed. The severity of the effects was dose-related, and males tended to show more hepatic cell changes than females. Changes seen at the 5 ppm level (0.25–0.5 mg/kg/day) were considered by the authors as ”minimal”; changes seen at the 50 ppm level (2.5–4.6 mg/kg/day) were slight, sometimes moderate; the authors do not comment about what they saw in the 10 ppm (0.5–0.9 mg/kg/day) group, presumably the results were intermediate to the doses above and below. The results from the kinetic studies revealed that accumulation of DDT in fat occurred at all dietary levels tested and that females stored more DDT than males; storage reached a

maximum at 19–23 weeks; age did not affect the rate of DDT-accumulation; about 50–75% of DDT stored in fat remained after a 1-month DDT-free diet, and 25% remained after 3 months.

Dose and end point used for MRL derivation: 0.05 mg/kg/day; liver effects.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose?
Yes

In this dietary study, the amount of DDT added to the food was measured, but the actual food consumption and body weights of the rats were not measured. The calculated value for the NOAEL on which the MRL is based ranges from 0.05 to 0.09 mg/kg/day depending on the food consumption values used to calculate the actual dose of DDT consumed. Using the most conservative estimates of food consumption, the NOAEL for 1 ppm DDT in food = 0.05 mg DDT/kg/day; this was the value used for calculating the MRL.

Calculation of Food Consumption Values (Three Models)

(1) Using EPA 1986d Reference Values

Using the EPA 1986d Reference Values for Risk Assessment, as was done in the 1994 edition of this toxicological profile, yields a NOAEL of 0.05 mg/kg/day equivalent to feeding 1 ppm DDT in the food. This reference considers the food consumption of an average rat to be 0.05 kg food/kg body weight/day (averaged over a lifetime) and the average weight of a rat to be 0.35 kg. No allometric equation is used for calculating the food factor. However, this reference recommends using a food consumption value of 0.09 mg/kg/day for a subchronic 90-day study.

NOAEL= 1 ppm in food = 1 mg/kg food
1 mg DDT/kg food x 0.05 kg food/kg body weight/day=0.05 mg/kg/day

Equivalents for other food concentrations used in this study, as calculated by using the EPA 1986d Reference Values for Risk Assessment:
5 ppm= 0.25 mg/kg/day
10 ppm= 0.5 mg/kg/day
50 ppm= 2.5 mg/kg/day

(2) EPA 1988g Method, Chronic Duration Average Body Weights

The NOAEL is 1 ppm of DDT in dietary study feeding Osborne-Mendel rats for 15–27 weeks (105–109 days). EPA 1988g has time weighted average body weights for male or female Osborne-Mendel rats for either of two study durations: subchronic (weaning to 90 days) and chronic (weaning to 730 days or 2 years). This study does not exactly fall into either of the intervals for which the time weighted average weights were calculated, but the convention is to pick the chronic category for studies

longer than 90 days. The risk assessment calculations recommended in EPA 1988g were used for calculating doses for all the dietary studies discussed in this toxicological profile.

$F= 0.056(W)^{0.6611}$ where F = kg food/day and W = body weight in kilograms

For chronic duration, the average body weight of male and female Osborne-Mendel rats is 0.452 kg, yielding an F=0.033 kg/food/day

NOAEL= 1 ppm in food = 1 mg/kg food
1 mg DDT/kg food x 0.033 kg food/day/0.452 kg body weight = 0.07 mg DDT/kg/day.

Equivalents for other food concentrations used in this study, as calculated by using the EPA 1986d Reference Values for Risk Assessment:
5 ppm= 0.4 mg/kg/day
10 ppm= 0.7 mg/kg/day
50 ppm= 3.7 mg/kg/day

(3) EPA 1988g Method, Subchronic Duration Average Body Weights

For subchronic duration (actually less than the duration of this study), the average body weight of male and female Osborne-Mendel rat 0.232 kg, yielding an F=0.0213 kg/food/day

NOAEL= 1 ppm in food = 1 mg/kg food
1 mg DDT/kg food x 0.0213 kg food/day/0.232 kg body weight = 0.09 mg DDT/kg/day

Equivalents for other food concentrations used in this study, as calculated by using the EPA 1986d Reference Values for Risk Assessment:
5 ppm= 0.5 mg/kg/day
10 ppm= 0.9 mg/kg/day
50 ppm= 4.6 mg/kg/day

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: NA

Was a conversion used from intermittent to continuous exposure?
No

Other additional studies or pertinent information that lend support to this MRL: In the Fitzhugh and Nelson (1947) study, 16 female Osborne-Mendel rats were fed a diet containing 1,000 ppm technical DDT for 12 weeks. Using EPA 1988g reference values for female body weight and subchronic food consumption, the diet provided approximately 96 mg DDT/kg/day. Sacrifices were conducted at cessation of dosing and at various intervals after a DDT-free period. Liver changes were similar to those seen in the Laug et al. (1950) study, although of increased severity, and were still present in rats killed after 2 weeks in a DDT-free diet. Minimal liver changes were apparent after 4–6 weeks of recovery, and complete recovery was seen after 8 weeks. Hepatic effects ranging from increased liver weights to cellular necrosis have been reported in animals after chronic exposure in the diet.

The Laug et al. (1950) study serves also as the basis for an oral RfD derived by EPA for DDT (IRIS 2001a). The Fitzhugh and Nelson (1947) study is considered supportive for the RfD.

Agency Contact (Chemical Manager): Obaid Faroon

APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical Name: DDT
CAS Number: 50-29-3
Date: September 2002
Profile Status: Third Draft Post Public
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: N/A
Species: N/A

An oral MRL for chronic-duration exposure to DDT was not derived because of the inadequacy of the available data on liver effects in animals to describe the dose-response relationship at low-dose levels. In a brief communication, Fitzhugh (1948) stated that histopathological lesions occurred in the liver of rats fed 10 ppm DDT in the diet for 2 years, but no experimental details were given, so the quality of the study cannot be evaluated. Using reference values for body weight and food consumption from EPA (1988), it can estimated that the 10 ppm dietary level provided DDT doses of approximately 0.7 mg/kg/day. This dietary level was the lowest level tested in the study, but was still higher than the lowest level resulting in hepatic effects in the Laug et al. 1950 study used for derivation of the intermediate-duration MRL.

Agency Contact (Chemical Manager): Obaid Faroon

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3, Section 3.10, "Interactions with Other Substances," and Section 3.11, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (3-1, 3-2, and 3-3) and figures (3-1 and 3-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 3-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 3-1).

(5) Species The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.5, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in Chapter 9 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 3-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 6

Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation

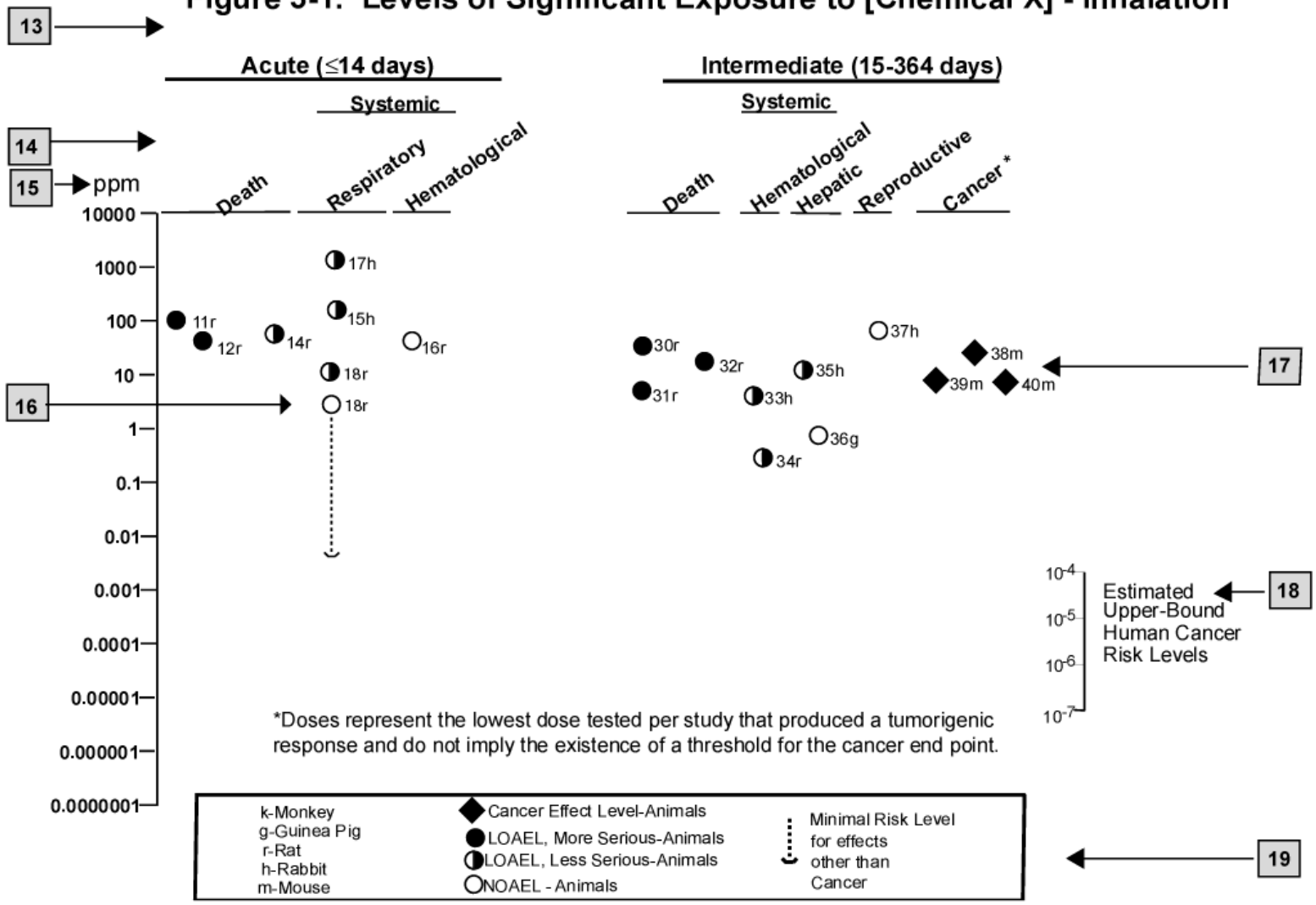
Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 6 INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 6 Systemic	9	9	9	9	9		9
4 6 18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					9		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 3-1.

12 6 [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACOEM	American College of Occupational and Environmental Medicine
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AOEC	Association of Occupational and Environmental Clinics
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotranferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

DOT/UN/NA/IMCO	Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	*Federal Register*
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LDH	lactic dehydrogenase
LH	luteinizing hormone
LT_{50}	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic

PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PID	photo ionization detector
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
RQ	reportable quantity
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
$	greater than or equal to
=	equal to
<	less than

#	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D

D. HEALTH EFFECTS IN WILDLIFE POTENTIALLY RELEVANT TO HUMAN HEALTH

Overview. The 1972 EPA decision to ban DDT for most crop uses in the United States was significantly influenced by a large body of scientific information indicating adverse health effects in wildlife (EPA 1975). The wildlife health effects that were considered by EPA in banning DDT were severe, including lethality of DDT in birds and fish and DDE-induced reproductive effects in birds, particularly eggshell thinning (EPA 1975). Wildlife may be regarded as sentinels for human health (NRC 1991). Although it is difficult to draw firm conclusions about human health from the toxicity observed in sentinel wildlife species in the environment, these observations have motivated more precise experiments with known doses of DDT, DDD, and DDE in both wildlife species and more traditional laboratory animal models, as well as identifying key health end points for epidemiological investigation. Wildlife field observations have also stimulated investigation of reproductive effects in mammalian models more directly relevant to humans and *in vitro* and mechanism of action studies that have resulted in the identification of some DDT isomers and metabolites as androgen antagonists and estrogen agonists. There have been a number of intriguing mechanistic studies of DDT isomers and metabolites in fish that relate to reproductive and developmental effects (Das and Thomas 1999; Faulk et al. 1999; Khan and Thomas 1998; Loomis and Thomas 1999; Sperry and Thomas 1999; Thomas 1999); these are discussed in Section 3.6.2, Mechanisms of Toxicity. Environmental monitoring studies have shown that DDT, DDE, and DDD are also highly persistent in the environment (see Section 6.3, Environmental Fate), and therefore, continue to present a potential health hazard both to humans and wildlife. It should not be forgotten that wildlife is part of the same broad ecological web as humans, and thus, toxic effects on wildlife ultimately affect the quality of human life as well.

Field observations of health effects in wildlife have strongly influenced the design of experimental studies. A high degree of causal uncertainty usually exists in field studies because wildlife species are frequently co-exposed to many other toxicants, such as other organochlorine pesticides and heavy metals, and are subjected to a variety of other uncontrolled and unknown stresses that may affect their health and confound an analysis of causation of a particular effect.

The purpose of this section is to provide a qualitative synopsis of health effects in terrestrial wildlife to address the potential concern that effects observed in wildlife that are attributable to DDT/DDE/DDD exposure may also occur in humans. The organization of Appendix D, Health Effects in Wildlife Potentially Relevant to Human Health, closely parallels that of Section 3.2, Discussion of Health Effects by Route of Exposure, to facilitate weight-of-evidence evaluations that may use both human health and wildlife toxicological effects data. The primary focus of this section is on experimental studies with known doses of DDT isomers and metabolites, but key observations from ecological field studies of wildlife have also been highlighted. These ecological field studies include observations of reproductive developmental effects in alligators living in contaminated Lake Apopka, Florida. No case reports of lethality in wildlife populations from exposure during DDT application were included because the exposure scenario is no longer relevant since the banning of DDT in 1972.

Where statistical significance of an observed effect was evaluated in a wildlife experimental study, statistical significance (p#0.05) was indicated in the text of this section using the term “significant”; if the term “significant” was not used in describing an observed effect, then no statistical evaluation is implied. This convention for this section is in contrast to the convention used in Section 3.2, Discussion of Health Effects by Route of Exposure, in which observed effects that are discussed in the text are usually assumed to be significant unless otherwise specified. Unfortunately, many experimental studies in wildlife used a small number of animals, making meaningful statistical tests of significance difficult. Study designs for

testing toxicity in more traditional laboratory animals have generally included larger samples sizes, facilitating tests of significance.

Experimental wildlife health effects information concerning DDT/DDE/DDD exposures was located for approximately 40 terrestrial species and on numerous end points. A large proportion of the information, however, is represented by a relatively small number of species and end points. The most heavily studied taxonomic group was birds, and among birds, the mallard/white Pekin duck (*Anas platyrhyncus*), Japanese quail (*Coturnix coturnix japonica*), domestic fowl, and ringed turtle dove (*Streptopelia risoria*) had the greatest representation. The most commonly reported end points were lethality, and neurological and reproductive end points. Of particular interest are those effects that were observed consistently across species and in spite of variability in exposure scenarios. The significant health effects most consistently reported were lethality (several taxa), hepatic (liver enzyme induction and liver damage in birds), endocrine (estrogenic effects in several taxa, and reduced thyroid weight and altered thyroid activity in birds), neurological (tremors in several taxa), reproductive (oviposition delay and eggshell thinning in birds), and developmental (reduced chick survival in birds, testicular feminization).

A hazard identification table (Table D-1) is provided so the reader may quickly scan the wildlife database for species or toxicological end points that are of particular interest. The table is divided into the following four sections based on species taxonomy: Wild mammals, reptiles, and amphibians; Birds—raptors, wading birds, water birds; Birds—gallinaceous birds; and Birds—passerines and nonpasserine ground birds. The organization of effect categories within each section of the table parallels that of the text so that more detailed information for particular table entries may be readily located. The specific toxicological effects reflected in the table under each effect category may vary between sections of the table, since specific end points may have been evaluated in certain species but not in others. Not every study mentioned in the text was reported in the table. Some studies that reported ambiguous results with respect to certain toxicological end points are included in the text, but no corresponding entry was made in Table D-1 for the ambiguous results. Not every significant effect reported in the text received a unique entry in Table D-1. Effects reported in different studies that would receive the same coding for a given species were all represented by a single entry in the table. Effects that were observed at high exposure levels but not at low exposure levels were entered once in the table as an observed effect. The individual isomers of DDT, DDE, and DDD, as well as technical-grades and unspecified mixtures, received unique codes in the table so that the reader may readily focus on compounds of particular interest. Since Table D-1 is intended for hazard identification, parenteral exposures were included, as well as oral, inhalation, and dermal routes that are more directly relevant to human environmental exposures. In contrast, the LSE tables in the previous section included only inhalation, oral, and dermal exposures.

Table D-1 is intended to be a visual aid to illustrate the wildlife database; it is not intended to be a stand-alone document. The reader should refer to the text for more detailed experimental design information and for information concerning the statistical significance of the observed effects.

D.1 Death

Based on the following analysis, the order of susceptibility to DDT-induced mortality appears to be amphibians>mammals>birds. Animals on restricted diets, such as migrating or nesting animals, are generally more sensitive to DDT-induced lethality than animals fed on nutritionally sufficient diets.

Mammals. Studies of DDT lethality in mammals are limited to bats and shrews, and generally attribute the cause of death to accumulation of DDT/DDE/DDD in the brain. In pipistrelle bats (*pipistrellus*), no mortality occurred after single oral doses of *p,p'*-DDT below 45 mg/kg body weight for up to 31 days postdosing, but 100% mortality occurred within 28 days in groups administered single doses of

$95 mg/kg body weight; the estimated LD_{50} was 63 mg/kg body weight (Jefferies 1972). A single oral dose of technical-grade DDT at 20 mg/kg body weight caused some mortality in big brown bats (*Eptesicus fuscus*), while $40 mg/kg body weight was 100% lethal (Luckens and Davis 1964). For 40 days, free-tailed bats (*Tadarida brasiliensis*) were fed mealworms that were raised in wheat bran containing 100 ppm *p,p'*-DDE; the treated bats lost body weight quicker and died sooner than an untreated control group during a postexposure starvation period (Clark and Kroll 1977). Among the 17 treated bats, a strong negative relationship was seen between *p,p'*-DDE residue in the brain and percent lipid in the carcass, suggesting that *p,p'*-DDE mobilized from fat will accumulate in the brain (Clark and Kroll 1977). Based on a review of the experimental literature and DDT/DDE/DDD residues in dead wild bats, Clark (1981) estimated that the minimum lethal concentrations are 12 ppm (w/w) DDT in the brain of the little brown bat (*Myotis liucifugus*), and 460 and 540 ppm DDE in the free-tailed bat and the little brown bat, respectively. The combined effect of starvation and exercise on the disposition of existing DDE body burden was evaluated in wild-captured free-tailed bats (Geluso et al. 1976). Median brain DDE in the starved-sedentary group was increased by a factor of 12.7 in young bats to 53.8 in older bats relative to the unstarved group, and median brain DDE in the starved-exercised group was increased by a factor of 43.2 in young bats to 123.1 in older bats over levels in the unstarved group, suggesting that bats may be particularly vulnerable to DDE-induced mortality during migration.

Dietary LC_{50} values in short-tailed shrews (*Blarina brevicauda*) for 14-day *p,p'*-DDT exposures ranged from 651 to 1,160 mg/kg diet when DDT was dissolved in oil prior to mixing in the diet, and ranged from 839 to 2,550 mg/kg diet when DDT was added as the powder (Blus 1978). The concentration of DDT in the brains of shrews that died was highly variable, and DDE residues were relatively low, suggesting that accumulation in the brain had little effect on mortality in shrews.

Amphibians. Lethality information in adult amphibians is limited to studies in the common frog (*Rana temporaria*) and the bullfrog (*Rana catesbeiana*). No mortality was seen in adult common frogs dosed twice weekly for 8 weeks with DDT (isomeric composition not specified) at 0.6 mg/kg, but in treated frogs that were not fed, 50% mortality was seen by the end of the exposure period (Harri et al. 1979). The LD_{50} in the adult common frog 20 days after a single oral administration of DDT (unspecified isomer) in gelatin capsules at unreported dose levels was estimated to be 7.6 mg/kg body weight; LD_{50} values at 3 and 4 days after the single oral administration were approximately 85 and 25 mg/kg, respectively (Harri et al. 1979). Mortality was seen in common frog tadpoles immersed for 1 hour in 1 or 10 ppm *p,p'*-DDT, but not in #0.1 ppm (Cooke 1970a). In the adult bullfrog, a 14-day oral LD_{50} of >2,000 mg/kg body weight was reported following a single oral administration of *p,p'*-DDT in gelatin capsules at unreported dose levels (U.S. Fish and Wildlife Service 1984).

Birds. Historically, observations of high mortality in local wild bird populations occurred coincidentally with application of DDT for pest control (EPA 1975). At the site of DDT application, local bird populations were acutely exposed by inhalation of airborne DDT, by ingestion of DDT residues on insects and other invertebrates such as earthworms, and by direct ingestion of DDT while preening. Several authors have postulated that high mortality may occur during times of stress, such as during nesting or during migration, when energy from fat stores is mobilized (EPA 1975). As fat stores are depleted, fat-stored and newly absorbed DDT could distribute to the brain; as in mammals, accumulation of high levels in the brain of birds is hypothesized to be lethal (EPA 1975). Since DDT was banned, the primary route of exposure to DDT compounds in wild bird populations has been in the diet through the food chain. Available experimental data on bird lethality indicate that DDT/DDE/DDD have moderate to low toxicity in birds after ingestion in the diet or from gavage administration (WHO 1989).

Acute LD_{50} values of orally administered DDT (unspecified isomeric composition) in 2-month-old Japanese quail (*Coturnix coturnix japonica*), *p,p'*-DDT in 4-month-old pheasant (*Phasianus colchicus*), technical-grade DDT in 6-month-old California quail (*Callipepia californica*), DDT (unspecified isomeric

composition) in 3-month-old Mallard ducks (*Anas platyrhyncus*), DDT (unspecified isomeric composition) in the rock dove (*Columba livia*), and *p,p'*-DDT in the adult sandhill crane (*Grus canadensis*) ranged from 595 mg/kg body weight in 6-month-old male California quail to >4,000 mg/kg body weight in male and female rock doves (U.S. Fish and Wildlife Service 1984). Dietary LC_{50} values for DDT (unspecified isomeric composition) ingestion ranged from 311 to 1,869 mg/kg diet after 5-day exposures in immature bobwhite quail (*Colinus virginianus*), Japanese quail, Mallard duck, and pheasant (U.S. Fish and Wildlife Service 1965, 1975). A dietary LC_{50} of 1,612 mg/kg diet for *p,p'*-DDT after a 5-day exposure was reported in 10-week-old clapper rails (*Rallus longirostris*) (Van Veltzen and Kreitzer 1975). Dietary LC_{50} values for technical-grade DDT after 5-day exposures in immature bobwhite quail, cardinal (*Richmondena cardinalis*), house sparrow (*Passer domesticus*), and blue jay (*Cyanocitta cristata*) ranged from 415 to 1,610 mg/kg diet (Hill et al. 1971). Dietary LC_{50} values for *p,p'*-DDT after 10-day exposures in immature Mallard ducks ranged from 1,202 to 1,622 mg/kg, and in adult Mallard ducks was 1,419 mg/kg (Friend and Trainer 1974a). The dietary LC_{50} values for DDT (unspecified isomeric composition) after acute (<10 days) exposure in immature bobwhite quail, pheasants, and Mallard ducks ranged from 500 to 1,000 mg/kg diet and in adult bobwhite and pheasants, ranged from 1,000 to 2,500 mg/kg diet; after intermediate (<100 days) exposure, LC_{50} values in immature birds ranged from 100 to 400 mg/kg diet and in adult birds (including Mallards) from >100 to 1,000 mg/kg diet (U.S. Fish and Wildlife Service 1963). In the red-winged blackbird (*Agelaius phoeniceus*), dietary LC_{50} values after acute (<10 days) and intermediate (<30 days) exposures to DDT (unspecified isomeric composition) were 1,000 and 500 mg/kg diet, respectively (U.S. Fish and Wildlife Service 1963). Dietary LC_{50} values for ingestion of *p,p'*-DDE (isomeric composition not specified) ranged from 825 to 3,570 mg/kg diet after 5-day exposures in immature bobwhite quail, Japanese quail, Mallard duck, and pheasant (U.S. Fish and Wildlife Service 1975). Acute (exposure duration not specified) oral LD_{50} values for DDD (unspecified isomeric composition) were 386, >700, and >2,000 mg/kg body weight in 3- to 4-month-old pheasants, 6-month-old California quail, and 3-month-old Mallard ducks (U.S. Fish and Wildlife Service 1984). Dietary LC_{50} values for technical-grade DDD ranged from 445 to 4,810 mg/kg diet after 5-day exposures in immature bobwhite quail, Japanese quail, pheasant, and Mallard ducks (U.S. Fish and Wildlife Service 1975).

Time to death in adult and immature bald eagles (*Haliaetus leucocephalus*) was inversely related to dietary technical-grade DDT level in the feed; birds fed 4,000 ppm died within 23 days, while birds at lower exposure levels survived for up to 112 days (Chura and Stewart 1967). All three bald eagles (adult and immature) fed 4,000 ppm technical-grade DDT in the diet died after at least 15 days of exposure, and one of two eagles fed 160 ppm technical DDT in the diet died after at least 76 days of exposure (Chura and Stewart 1967; Locke et al. 1966). Groups of 10–30 Mallard ducks (5-day-old, 30-day-old, and adult ducks) were fed *p,p'*-DDT at 250–2,000 ppm for 10 days in a diet specially formulated to provide adequate nutrition, but minimize fat formation (Friend and Trainer 1974a); onset and mean time to mortality occurred earlier in younger ducks compared to older ducks, although mortality was seen in all age groups. Survival times up to 49 and 29 days were seen in house sparrows fed 200 and 300 ppm DDT (unspecified isomer), respectively, in chick starter mash (Bernard 1963). DDT (isomeric composition not specified) provided in drinking water (12% solution) or as residue in earthworms sprayed twice daily with a 12% solution (DDT residue concentration in earthworms was not reported) caused 100% mortality in wild-captured adult house sparrows within 17 days of the initial exposure (Boykins 1967).

Liver DDE was 128 and 253 ppm in two male kestrels that died compared to a mean level of 24 ppm in surviving males, and brain DDE residues were 212 and 301 ppm in the two birds that died compared to a mean level of 15 ppm in surviving males (Porter and Wiemeyer 1972). DDT and DDE residue levels in the brains of ducks that died were between 6 and 17 times the levels seen in ducks that survived to 10 days postexposure in groups of 10–30 Mallard ducks (5-day-old, 30-day-old, and adult ducks) provided with *p,p'*-DDT at 250–2,000 ppm for 10 days in a diet specially formulated to provide adequate nutrition, but minimize fat formation (Friend and Trainer 1974a); DDT residue levels were generally

higher in the younger ducks than in older ducks. In contrast, DDE levels in the brains of cowbirds (*Molothrus ater*) that survived 8–12 days of dietary exposure to 500 ppm *p,p'*-DDT were higher than brain DDE levels in birds that died during exposure, suggesting that brain DDE was not the principle factor inducing mortality in cowbirds (Stickel et al. 1966).

Observations of mortality were reported in several other studies. Mortality was observed in 2/12 male kestrels (*Falco sparverius*), one adult and one yearling, exposed for at least 14 months on diets containing 2.8 ppm *p,p'*-DDE (Porter and Wiemeyer 1972). There was no significant increase in mortality rate in barn owls (*Tyto alba*) fed 3 ppm DDE (isomeric composition not reported) in the diet for 2 years compared with controls (Mendenhall et al. 1983); however, two DDE-treated females died when they were unable to lay eggs that were "virtually shell-less." Mortality occurred in 6–29% of female pheasants (*Phasiannus colchicus*) and 33–100% of male pheasants fed $100 ppm technical-grade DDT for at least 22 days; no deaths occurred in birds fed 10 ppm for up to 101 days (Azevedo et al. 1965). Survival of Japanese quail administered up to 50 ppm DDT (unspecified isomeric composition) was reportedly comparable to control group survival throughout a 3-generation reproductive toxicity assay (Shellenberger 1978). *o,p'*- and *p,p'*-DDT increased pentobarbital-induced mortality rates in Japanese quail when administered in the diet at 100 ppm for 2–14 days prior to a single intramuscular injection of pentobarbital (Bitman et al. 1971). Mortality in Japanese quail (*Coturnix coturnix japonica*) was significantly exposure-related in birds fed diets containing between 700 and 1,600 ppm *p,p'*-DDT for 20 days; factors that affected weight, such as restricted diet, sex, strain, and breeding condition, also affected susceptibility to DDT intoxication—heavier birds were less susceptible than lighter, more stressed birds (Gish and Chura 1970). No effect on hen mortality was seen in laying bobwhite quail (*Colinus virginianus*) administered up to 20 mg/bird of DDT (unspecified isomeric composition) every other day during a 4-week exposure period (Wilson et al. 1973). *p,p'*-DDT provided in the diet caused mortality in Bengalese finches (*Lonchura striata*) at 84 ppm (within 46 days) and 168 ppm (within 35 days), but not at #42 ppm (Jefferies and Walker 1966).

D.2 Systemic Effects

Respiratory Effects. No experimental studies were located regarding respiratory effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD.

Birds. No gross lung lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days or 500 ppm for up to 23 days (Azevedo et al. 1965).

Cardiovascular Effects. No experimental studies were located regarding cardiovascular effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD.

No cardiovascular effects were observed in birds after dietary exposures to DDT/DDE/DDD, but capsular bolus administration of DDT or DDE resulted in changes in heart morphology and function that may be interpreted to be secondary to thyroid effects. Cardiovascular lesions were limited to a single observation of poor heart muscular tone at relatively high intermediate duration exposures.

Birds. Heart weights were not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) exposed by daily oral administrations of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDE, and 15 mg *p,p'*-DDD for 10 weeks (Greichus et al. 1975); doses were injected into the first fish fed to the birds each day. Heart weights were not significantly decreased in double-crested cormorants (*Phalacrocorax auritus*) fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Heart weight was increased in homing pigeons (*Columba livia*) administered 3 mg/kg/day of *p,p'*-DDT by capsule for 8 weeks, but heart weights showed exposure-related decreases at doses of 6–54 mg/kg/day, with an overall significant decreasing trend in heart weight with dose (Jefferies et al.

1971). A significant dose-related decreasing trend in heart weight was also seen in pigeons administered daily doses of 18–72 mg/kg/day of *p,p'*-DDE by capsule for 8 weeks (Jefferies and French 1972). A dose-related increase in relative heart weight was observed in Bengalese finches (*Lonchura striata*) fed up to 0.3 mg/kg/day of *p,p'*-DDT for at least 15 weeks (Jefferies 1969). Jefferies (1969) suggested that the significant increasing trend in heart weight with dose observed in Bengalese finches at low doses was due to DDT-induced hyperthyroidism. In a later study in pigeons, Jefferies et al. (1971) refined the hypothesis, suggesting a biphasic heart response to changes in thyroid activity with increasing dose. They postulated that increased heart weight in homing pigeons at low, intermediate-duration oral exposures was due to a hyperthyroid condition, while a dose-related decrease in heart weights at higher oral doses in pigeons occurred due to hypothyroidism. The hypothesis of a hyper-hypothyroidism continuum with increasing dose was supported by observations (discussed below) that pulse rate and ventricular S-wave amplitude peaked at low doses in homing pigeons, with a dose-related decline of both parameters at higher dose levels. Further data supporting the hypothesis of biphasic thyroid activity in pigeons is provided in an observation of biphasic metabolic rate (Jefferies and French 1971), increased thyroid weights with decreased thyroidal colloid (Jefferies and French 1969, 1971), and increased adrenal weights (Jefferies et al. 1971). These effects are discussed further below under Endocrine Effects.

Pulse rate was increased above controls at all dose levels tested in the homing pigeon fed *p,p'*-DDT by capsule for 3 or 6 weeks; pulse rate peaked at 18% above controls at 3 mg/kg/day, and showed a decreasing trend with dose level at 6–36 mg/kg/day (Jefferies et al. 1971). Bengalese finches showed an exposure-related increase in pulse rate at oral doses of *p,p'*-DDT up to 11.7 mg/kg/day (Jefferies et al. 1971).

The amplitude of the ventricular beat in homing pigeons peaked at 3 mg/kg/day after 3- or 6-week oral exposures and showed a significant exposure-related decrease at exposure levels up to 36 mg/kg/day (Jefferies et al. 1971). In Bengalese finches, there was no clear effect on the amplitude of the ventricular beat at oral dose levels up to 11.7 mg/kg/day for up to 6 weeks of exposure (Jefferies et al. 1971).

No gross heart lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days or 500 ppm for up to 23 days (Azevedo et al. 1965). Hearts in two homing pigeons receiving daily doses of $36 mg/kg/day *p,p'*-DDE (but not at #18 mg/kg/day) for 8 weeks were "flaccid, with thin musculature" (Jefferies and French 1972).

Gastrointestinal Effects. No experimental studies were located regarding gastrointestinal effects in wildlife from exposure to DDT/DDE/DDD.

Hematological Effects. No experimental studies were located regarding hematological effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD. In experimental studies in birds, hematological effects were observed inconsistently.

Hematological effects were inconsistently observed in birds after oral and subcutaneous exposures to DDT compounds. Bird hematology appeared to be more sensitive to gavage exposure than to dietary exposure. Relatively high, acute gavage exposure in crowned guinea hens resulted in decreased hemoglobin, erythrocyte count, and hematocrit, while no effect on hemoglobin content or hematocrit were observed in Japanese quail and cormorants exposed in the diet for an intermediate duration. Alternative hypotheses explaining the decreases in red blood cell indices after bolus doses include a direct disruption of erythrocyte membranes by lipophilic DDT, a reduced erythrocyte viability from nuclear metabolic interference, an inhibition of erythrocyte proliferation in hematopoetic tissue, or an estrogenic inhibitory effect on red blood cells (Fourie and Hattingh 1979). Intermediate-duration dietary exposures in bobwhite quail and ringed turtle doves resulted in increased and decreased hematocrits, respectively.

Except for decreased red blood cell count in cockerels (young male chickens; young roosters; domestic fowl), parenteral exposures in domestic fowl showed no effects in hematological parameters.

Birds. Blood hemoglobin was not significantly affected in double-crested cormorants (*Phalacrocorax auritus*) fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Hemoglobin was significantly decreased in crowned guinea fowl (*Numida meleagris*) after an assumed daily gavage dose of 75 mg/kg body weight (exposure units were ambiguously reported) of technical-grade DDT on 5 consecutive days (Fourie and Hattingh 1979), while a 12-week dietary exposure to up to 100 ppm *p,p'*-DDE did not significantly affect hemoglobin concentration in Japanese quail (*Coturnix coturnix japonica*) (Dieter 1974). No effect on hemoglobin level was seen in chickens administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days for a cumulative dose of 2–3 g/chicken (Burlington and Lindeman 1952).

Erythrocyte count was significantly decreased in crowned guinea fowl after an assumed daily gavage dose of 75 mg/kg body weight (exposure units were ambiguously reported) of technical-grade DDT on 5 consecutive days (Fourie and Hattingh 1979). No effect on erythrocyte count was seen in chickens administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days for a cumulative dose of 2–3 g/chicken (Burlington and Lindeman 1952). Erythrocyte count was reduced compared to controls by an average of 17.8% in White Leghorn cockerels injected with DDT (unspecified isomer) subcutaneously for between 60 and 89 days at dose levels that increased from 15 to 300 mg/kg/day during the exposure period (Burlington and Lindeman 1950).

Hematocrit was not significantly affected in double-crested cormorants fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Hematocrit was significantly decreased in crowned guinea fowl (*Numida meleagris*) after an assumed daily gavage dose of 75 mg/kg body weight (exposure units were ambiguously reported) of technical-grade DDT on 5 consecutive days (Fourie and Hattingh 1979). A 12-week dietary exposure to up to 100 ppm *p,p'*-DDE did not significantly affect hematocrit in Japanese quail (Dieter 1974). Hematocrit was unaffected compared to controls in White Leghorn cockerels injected with DDT (unspecified isomer) subcutaneously for between 60 and 89 days at dose levels that increased from 15 to 300 mg/kg/day during the exposure period (Burlington and Lindeman 1950). Hematocrit was significantly increased in bobwhite quail (*Colinus virginianus*) fed diets containing from 10 (lowest level tested) to 150 ppm technical-grade DDT for several months (Lustick et al. 1972; Peterle et al. 1973). The increased hematocrit in bobwhite quail was hypothesized to be a compensatory increase in relative red blood cell volume related to increased metabolic oxygen demand caused by a DDT-induced increase in thyroxin secretion by the thyroid; the study confirmed increased uptake of I^{131} by the thyroid in DDT-exposed birds, indicating increased thyroid activity (Lustick et al. 1972; Peterle et al. 1973). Significantly decreased hematocrit was seen in ringed turtle doves (*Streptopelia risoria*) fed a diet containing 200 ppm (but not at #20 ppm) of DDE (unspecified isomeric composition) for 8 weeks (Heinz et al. 1980). The decrease in hematocrit observed in ringed turtle doves occurred only at a dietary exposure level of DDE greater than those used to administer DDT in the bobwhite quail study.

Musculoskeletal Effects. No experimental studies were located regarding musculoskeletal effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD.

Birds. No gross skeletal muscle lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days or 500 ppm for up to 23 days (Azevedo et al. 1965).

Calcium uptake in bone (which is mediated by estradiol) was significantly reduced (compared to controls) at 8 days postmating in ringed turtle doves (*Streptopelia risoria*) fed diets containing 10 ppm *p,p'*-DDT

for 3 weeks prior to mating, but not in birds allowed to complete their clutch (Peakall 1970). "Extremely soft skulls" were observed in homing pigeons (*Columba livia*) that died within 35 days of oral exposure to 72 mg/kg/day of *p,p'*-DDE in capsules (Jefferies and French 1972).

Hepatic Effects. No experimental studies were located regarding hepatic effects in reptiles or amphibians from exposure to DDT/DDE/DDD.

DDT and DDE consistently induced hepatic microsomal enzyme activity in six species of birds after oral exposures; mixed results were obtained in five studies in Japanese quail. Five of the studies showing enzyme induction indicated that the increased hepatic enzyme activity significantly accelerated the breakdown of steroid hormones, including progesterone and estrogen in females and testosterone in males, potentially affecting hormone balance. No effect on liver weights was observed in birds orally administered DDT alone, but significantly increased liver weights were seen in three species fed DDE alone, probably due to hepatic enzymatic induction. Decreased liver weights were observed in two piscivorous species after oral exposure to a mixture of DDT/DDE/DDD; the authors did not speculate on the cause of the decrease in liver weights. Increased activity of liver enzymes in the blood was consistently observed in three species, indicating liver cell damage (which causes the enzymes to leak out from the cells), and microscopic lesions of the liver were observed in two species. With few exceptions, liver effects are a consistent, albeit nonspecific, indicator of DDT/DDE/DDD toxicity in birds, and induction of liver-mediated metabolism may affect steroid hormone balance.

Mammals. Short-tailed shrews showed liver weights that were not significantly different from controls after consuming an earthworm diet containing an average of 16.6 ppm DDT for 3 weeks (unspecified isomeric composition) (Braham and Neal 1974).

Birds. The hepatic microsomal mixed function oxidase system was significantly induced in puffins administered approximately 6 mg DDE (unspecified isomer)/bird for 16–21 days before sacrifice by decapitation (Bend et al. 1977). Hepatic microsomal ethylmorphine N-demethylase activity was significantly increased in kestrels (*Falco sparverious*) fed diets containing 10 ppm DDE (unspecified isomeric composition) for 5 years (Gillett et al. 1970). No effect on hepatic microsomal protein, but significantly increased hepatic P-450 activity, were seen in domestic chicken hens orally administered 40 mg/hen of technical-grade DDT for 5 days (Chen et al. 1994).

Japanese quail (*Coturnix coturnix japonica*) showed increased pentobarbital-induced sleeping time when fed each of *o,p'*- and *p,p'*-isomers of DDT, DDE, and DDD separately at 100 ppm in the diet for up to 14 days (Bitman et al. 1971), indirectly suggesting that the DDT compounds inhibited the hepatic microsomal enzymes that metabolize pentobarbital. Gillett et al. (1970) reported significantly decreased hepatic microsomal epoxidase activity in Japanese quail fed up to 100 ppm DDT (unspecified isomeric composition) for 27 weeks. Another study provided some evidence that 28-day dietary exposure to 50 ppm of *p,p'*-DDT, DDE, and DDD altered (causing both increases and decreases) hepatic enzymatic activity in Japanese quail (Bunyan et al. 1970), while yet another study indicated increased hepatic microsomal P-450 levels without a significant change in microsomal protein content in Japanese quail fed diets of up to 100 ppm *p,p'*-DDE for 21 days (Bunyan and Page 1973; Bunyan et al. 1972). Liver oxidative enzyme activity was not affected in female Japanese quail fed diets containing up to 30 ppm technical-grade DDT for 107 days (Kenney et al. 1972).

Metabolism of 17β-estradiol was significantly increased in the hepatic microsomal fraction obtained from domestic fowl hens fed diets containing 300–1,200 ppm technical-grade DDT for 7 to 21 days (Britton 1975). Hepatic microsomal extracts from female bobwhite quail (*Colinus virginianus*) fed a diet containing 5 ppm technical-grade DDT for 30–70 days produced a significantly greater conversion of ^{14}C-labelled progesterone to metabolites *in vitro* than microsomal extracts from untreated female quail;

microsomal enzyme extracts from DDT-treated male quail did not produce a significant increase in mean testosterone conversion *in vitro* (Lustick et al. 1972; Peakall 1967; Peterle et al. 1973). Metabolism of both testosterone and progesterone was significantly increased over control levels in liver microsomal fractions obtained from male and female White King pigeons, respectively, after a 1-week dietary exposure to 10 ppm *p,p'*-DDT (Peakall 1967). Similarly, estradiol metabolism was significantly increased in liver microsomal fractions obtained from ringed turtle doves (*Streptopelia risoria*) fed 10 ppm of technical-grade DDT in the diet for 3 weeks (Peakall 1969). Hepatic enzyme metabolism of estradiol was significantly increased at 8 days postmating in ringed turtle doves fed diets containing 10 ppm *p,p'*-DDT for 3 weeks prior to mating, but not in birds allowed to complete their clutch (Peakall 1969, 1970).

Relative liver weights were significantly depressed in white pelicans (*Pelecanus erythrorhynchos*) administered a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE for 10 weeks (Greichus et al. 1975). Liver weights were significantly decreased in double-crested cormorants (*Phalacrocorax auritus*) fed diets containing $5 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Liver weights and yield of microsomal protein were not significantly affected in puffins administered approximately 6 mg DDE (unspecified isomer)/bird for 16–21 days before sacrifice by decapitation (Bend et al. 1977). In Japanese quail, relative liver weights were significantly increased after 12 weeks on a diet containing $25 ppm *p,p'*-DDE (Dieter 1974). In other studies with Japanese quail, liver weights were not significantly affected by nine oral administrations of 10 mg *o,p'*-DDT over 3 weeks (Cooke 1970b) or by dietary exposure to up to 100 ppm of the *p,p'*- isomers of DDT, DDE, and DDD for 28 days (Bunyan et al. 1970, 1972). Liver weight and microsomal protein were not significantly different from controls in Japanese quail fed diets containing up to 100 ppm DDT (unspecified isomeric composition) for 27 weeks (Gillett et al. 1970). Significantly increased relative liver weights were seen compared to controls in bobwhite quail fed diets containing 50–150 ppm technical-grade DDT for 30–70 days (Lustick et al. 1972). No consistent effect on chicken liver weights was seen in birds fed between 400 and 800 ppm *p,p'*-DDT in the feed for 2–6 weeks (Glick 1974). No significant effects on liver weights or hepatic microsomal protein levels were seen in domestic fowl hens orally administered 40 mg/hen of technical-grade DDT for 5 days (Chen et al. 1994). No effect on liver weights was seen in redstarts (*Phoenicurus phoenicurus*) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974). Significantly increased liver weights were seen in ringed turtle doves fed diets containing $20 ppm DDE (unspecified isomeric composition) for 8 weeks (Heinz et al. 1980). A significant dose-related increase in liver weight was seen in homing pigeons (*Columba livia*) administered daily doses of *p,p'*-DDE for 8 weeks (Jefferies and French 1972) or *p,p'*-DDT for 42 days (Jefferies and French 1969) by capsule at between 18 and 72 mg/kg/day.

Plasma liver enzyme activities (creatinine kinase, aspartate aminotransferase, cholinesterase, fructose-diphosphate aldose, and lactate dehydrogenase) were significantly increased in Japanese quail, suggesting liver damage at exposure levels of $5 ppm *p,p'*-DDE in the diet for 12 weeks (Dieter 1974). Crowned guinea fowl (*Numida meleagris*) showed significantly increased hepatic enzyme activities in blood collected after 5 days of oral exposure to approximately 75 mg/kg technical-grade DDT (dose was ambiguously reported) (Fourie and Hattingh 1979). Significantly increased plasma aspartate aminotransferase was seen in ringed turtle doves fed diets containing 200 ppm (but not #20 ppm) of DDE (unspecified isomeric composition) for 8 weeks (Heinz et al. 1980).

Centrilobular liver degeneration was seen in 6-day-old chickens within 2 days of a single intraperitoneal injection with 0.25 mmol *o,p'*-DDD, and increased in severity over the next several days (Jönsson et al. 1994). No gross liver lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days or 500 ppm for up to 23 days (Azevedo et al. 1965). Dose-related "marked" liver hypertrophy was observed in homing pigeons orally administered capsules

containing *p,p'*-DDT at doses ranging from 3 to 54 mg/kg/day over a 17-week period (Jefferies and French 1971).

Daily oral exposure of homing pigeons to *p,p'*-DDT in capsules ($36 mg/kg/day) for up to 8 weeks resulted in a significant decrease in hepatic vitamin A storage (Jefferies and French 1971) (vitamin A is a fat-soluble compound that is essential for normal night vision, health of epithelial cells, and normal growth of bones and teeth, and is stored in the liver). The authors suggested that the reduced liver vitamin A was related to DDT-induced hypothyroidism, as evidenced also by reduced body temperature and oxygen consumption. In contrast, a lower dose of DDT (3 mg/kg/day), which caused hyperthyroidism, significantly increased hepatic vitamin A storage. Liver vitamin A was significantly increased in white pelicans administered a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE injected into the first fish fed to the birds each day over a 10-week period (Greichus et al. 1975). Liver carotene (a vitamin A precursor converted to vitamin A in the liver) was not significantly different from controls (Greichus et al. 1975). Liver vitamin A content was significantly decreased in double-crested cormorants fed diets containing $5 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). In this case, liver carotene levels were also not significantly affected. Thyroid status was not evaluated in the studies of Greichus and coworkers.

Liver glycogen was marginally (not significantly) decreased and liver lipid levels were significantly increased in bobwhite quail fed diets containing 100 ppm technical-grade DDT for 10 weeks (Haynes 1972).

Serum lipid was not significantly affected in Japanese quail either by four consecutive daily intramuscular injections with 5 mg *o,p'*-DDT or by nine oral administrations of 10 mg/bird of *o,p'*-DDT over 3 weeks (Cooke 1970b). Crowned guinea fowl showed significantly increased serum cholesterol compared to pre-exposure levels after a 5-day oral exposure to approximately 75 mg/kg/day technical-grade DDT (Fourie and Hattingh 1979).

Significantly depressed serum protein was observed in white pelicans (*Pelecanus erythrorhynchos*) administered daily oral doses of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE for 10 weeks (Greichus et al. 1975). Blood total protein was not significantly affected in double-crested cormorants fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Plasma protein was not significantly affected in crowned guinea fowl after an assumed daily gavage dose of 75 mg/kg body weight (exposure units were ambiguously reported) of technical-grade DDT on 5 consecutive days (Fourie and Hattingh 1979). No effects on plasma protein level was seen in cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days starting on the 8th day posthatch; daily dose was unreported, but cumulative dose was 2–3 g/cockerel (Burlington and Lindeman 1952). No effects on fibrinogen level or prothrombin time were seen in blood of cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days for a cumulative dose of 2–3 g/cockerel (Burlington and Lindeman 1952). Prothrombin is formed and stored in the liver; in the presence of thromboplastin and calcium, it is converted into thrombin, which, in turn, converts fibrinogen to fibrin for blood coagulation.

Renal Effects. No experimental studies were located regarding renal effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD.

Birds. No renal effects were observed in experimental studies in birds. Kidney weights were not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) exposed by daily oral administrations of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE for 10 weeks (Greichus et al. 1975). No effect on kidney weights was seen in redstarts (*Phoenicurus*

phoenicurus) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974).

No gross kidney lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days, or 500 ppm for up to 23 days (Azevedo et al. 1965).

Endocrine Effects. No experimental studies were located regarding endocrine effects in wild mammals from exposure to DDT/DDE/DDD.

Thyroid weights in birds were consistently elevated in six studies of dietary or capsular DDT exposures. Thyroid function in birds appeared to be biphasic after dietary exposure, showing decreased I^{131} uptake or no change in two studies at #25 ppm DDT, and increased I^{131} uptake in four studies at $100 ppm. The opposite pattern appears to have occurred in birds administered bolus doses of DDT in capsules, with hyperthyroidism occurring at relatively low bolus doses and hypothyroidism at high doses. The incidence of lesions in thyroid tissue increased with increasing dosage; thus, the hypothyroidism observed at high bolus oral doses may be due to degenerative changes in the thyroid. Similar degeneration may not occur at high dietary concentrations because dietary exposure occurs relatively gradually over time and may not result in blood DDT levels high enough to damage thyroid tissue. Gross adrenal morphology was unaffected by oral exposure to DDT, but histological examination showed changes in the cortico-medullary ratio in birds fed DDT in the diet and degenerative changes in adrenals of birds exposed parenterally. Adrenal function has not been directly evaluated in wildlife species. Estrogenic changes have been reported in birds, reptiles, and amphibians after parenteral exposure to *o,p'*-DDT, and reduced phallus size and altered sex ratio at hatching have been reported in reptiles exposed *in ovo* to DDE. See Section 3.6.2, Mechanisms of Toxicity, for further discussion of estrogenicity of DDT/DDE/DDD in laboratory animals and *in vitro*.

Reptiles. Significantly elevated plasma vitellogenin, suggesting estrogenic activity, was induced in adult male red-eared turtles (*Trachemys scripta*) intraperitoneally injected with *o,p'*-DDT at either 1 or 250 µg/g/day for 7 days. The amounts of vitellogenin produced were significantly lower than those seen in turtles injected with 1 µg/g/day 17β-estradiol (Palmer and Palmer 1995). Vitellogenin is a protein precursor of egg proteins that is produced in the liver of oviparous and ovoviviparous species and is found in measurable quantities in blood; production is stimulated by estrogens in the blood that are produced in the ovary; thus, vitellogenin is normally absent in the blood of males (Hadley 1984).

Amphibians. Plasma vitellogenin was significantly increased in adult male African clawed frogs (*Xenopus laevis*) intraperitoneally injected with *o,p'*-DDT at either 1 or 250 µg/g/day for 7 days (Palmer and Palmer 1995), suggesting estrogenic activity. However, the amounts of plasma vitellogenin induced were significantly lower than those seen in frogs injected with 1 µg/g/day 17β-estradiol (Palmer and Palmer 1995).

Birds. Thyroid weights were significantly increased in an exposure-related manner in juvenile male Mallards (*Anas platyrhyncus*) fed technical-grade DDT at 2.5–250 ppm in the diet for 30 days (Peterle et al. 1973). Thyroid glands were significantly enlarged in bobwhite quail (*Colinus virginianus*) fed technical-grade DDT in the diet at 500 ppm (but not at #50 ppm) for at least 3 months (Hurst et al. 1974). Bobwhite quail fed on diets containing 100 ppm (but not 10 ppm) technical-grade DDT for at least 13 weeks showed significantly increased thyroid weights relative to controls (Lustick et al. 1972). Significantly increased relative thyroid weight was seen in bobwhite quail held for 1 week at -18 EC after being fed 100 ppm technical-grade DDT for several months, but not in controls or birds fed 10 ppm (Peterle et al. 1973). A significant treatment-related increase in relative thyroid weight was seen in homing pigeons (*Columba livia*) fed *p,p'*-DDT or *p,p'*-DDE in capsules every other day over a 42- to 56-day period at 18–72 mg/kg/day (Jefferies and French 1969, 1972). Another experiment by Jefferies

and French (1971) confirmed the finding of increased thyroid weight in homing pigeons in response to daily oral exposure to *p,p'*-DDT (3–36 mg/kg/day) for up to 8 weeks.

Uptake of I^{131} into the thyroid was decreased (not significantly) in juvenile male mallards (*Anas platyrhyncus*) fed 2.5 or 25 ppm technical-grade DDT in the diet for 30 days, and a significantly increased uptake of I^{131} into the thyroid was seen in birds at 250 ppm DDT (Peterle et al. 1973). Thyroid uptake of I^{131} was significantly increased in bobwhite quail fed 500 ppm technical-grade DDT in the diet for 1–3 months, but not after 4 months of treatment (Hurst et al. 1974). Bobwhite quail fed diets containing 100 ppm (but not 10 ppm) technical-grade DDT for at least 13 weeks showed significantly increased thyroid activity as measured by I^{131} uptake relative to controls (Lustick et al. 1972). In another experiment, significantly increased I^{131} uptake into the thyroid was seen in bobwhite quail held for 1 week at -18 EC after being fed 100 ppm technical-grade DDT for several months, but not in controls or birds fed 10 ppm (Peterle et al. 1973). Jefferies and French (1971) found that body temperature and oxygen consumption were decreased in a dose-related manner in homing pigeons (*Columba livia*) in response to daily oral exposure to *p,p'*-DDT in capsules (3–36 mg/kg/day) for up to 8 weeks, and that significantly increased hepatic vitamin A storage (suggesting hyperthyroidism) was seen in the low-dose group, while significantly decreased hepatic vitamin A storage (suggesting hypothyroidism) was seen in the high-dose group; the authors noted that hypothyroidism has been associated with eggshell thinning in birds.

The incidence of hyperplasia of the thyroid follicular epithelium was increased in treated birds, and follicular colloid was decreased, in homing pigeons fed 18–72 mg/kg/day of *p,p'*-DDT or *p,p'*-DDE in capsules every other day over a 42- to 56-day period (Jefferies and French 1969, 1972). Another experiment by Jefferies and French (1971) confirmed the finding of hyperplasia and decreased follicular colloid in homing pigeons in response to daily oral exposure to *p,p'*-DDT (3–36 mg/kg/day) for up to 8 weeks.

Adrenal weight was unaffected in juvenile male Mallards fed technical-grade DDT at 2.5–250 ppm in the diet for 30 days (Peterle et al. 1973). Adrenal weights were not significantly affected in domestic chickens by a 6-week dietary exposure to *p,p'*-DDT at 400 or 800 ppm in the feed (Glick 1974). Adrenal gland weight was not significantly affected in bobwhite quail fed technical-grade DDT in the diet at 500 ppm (but not at #50 ppm) for at least 3 months (Hurst et al. 1974). Adrenal weights were not significantly affected in bobwhite quail fed diets containing up to 150 ppm technical-grade DDT for up to 242 days (Lehman et al. 1974). No significant effect was seen on adrenal weight in bobwhite quail at exposure levels up to 150 ppm of technical-grade DDT in the diet for several months (Peterle et al. 1973). A significant dose-related increase in adrenal weights was observed in homing pigeons fed *p,p'*-DDT in the diet at estimated dose rates of up to 54 mg/kg/day for 8 weeks (Jefferies et al. 1971) and in homing pigeons administered *p,p'*-DDE in capsules at 18–72 mg/kg/day for 8 weeks (Jefferies and French 1972).

Adrenal cortico-medullary ratio was unaffected in juvenile male mallards fed technical-grade DDT at 2.5–250 ppm in the diet for 30 days (Peterle et al. 1973). An exposure-related increase in adrenal cortical-medullary ratio was observed in bobwhite quail at exposure levels up to 150 ppm of technical-grade DDT in the diet for several months (Peterle et al. 1973); the authors speculated that this alteration of the cortico-medullary ratio may induce a reduced production and release of adrenaline compared to levels of corticosteroids. In bobwhite quail fed diets containing up to 150 ppm technical-grade DDT for up to 242 days, there was a significant exposure-related increase in the cortico-medullary ratio of adrenal gland (Lehman et al. 1974). Focal vacuolation and pycnotic nuclei were seen in adrenal interrenal cells of 6-day-old chickens within 1 week of a single intraperitoneal injection with 0.25 mmol *o,p*'-DDD (Jönsson et al. 1994).

Plasma corticosterone levels were unaffected in juvenile male mallards (*Anas platyrhyncus*) fed technical-grade DDT at 2.5–250 ppm in the diet for 30 days (Peterle et al. 1973).

No studies were located that directly evaluated for effects to the pancreas; however, a few studies measured blood sugar levels. Crowned guinea fowl showed significantly decreased serum blood sugar compared to pre-exposure levels after a 5-day oral exposure to approximately 75 mg/kg/day technical-grade DDT (Fourie and Hattingh 1979). No effect on blood sugar level was seen in cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days starting on the 8th day posthatch; daily dose was unreported, but cumulative dose was 2–3 g/chicken (Burlington and Lindeman 1952).

Dermal Effects. No experimental studies were located regarding dermal effects in wildlife from exposures to DDT/DDE/DDD.

Ocular Effects. No experimental studies were located regarding ocular effects in wildlife from exposures to DDT/DDE/DDD.

Body Weight Effects. No experimental studies were located regarding body weight effects in reptiles from exposure to DDT/DDE/DDD.

Body weight loss or reduced body weight gain were observed in mammalian, avian, and amphibian species in response to DDT/DDE/DDD exposures. Birds were most intensively studied, and among birds, raptors, passerines, and nonpasserine ground birds were more sensitive to DDT/DDE/DDD exposures with respect to body weight changes than gallinaceous birds. Body weight loss has been associated with hyperthyroidism, and weight gain has been associated with hypothyroidism (Jefferies 1969); see further discussion of DDT-induced thyroid effects under Endocrine, above. No clear patterns in body weight changes were evident regarding relative sensitivities based on particular DDT compound or route of exposure.

Mammals. Free-tailed bats (*Tadarida brasiliensis*) fed diets containing 107 ppm DDE (unspecified isomeric composition) showed significantly decreased body weight gain compared to controls after a 40-day exposure period (Clark and Kroll 1977). Body weights were generally not significantly affected in short-tailed shrews (*Blarina brevicauda*) fed DDT (unspecified isomeric composition) in the diet for 7, 14, or 17 days (Blus 1978); exposure levels were not reported, but were sufficiently high to calculate LC_{50} levels of $651 mg/kg diet. Another study in short-tailed shrews also reported body weights comparable to controls after consuming an earthworm diet containing an average of 16.6 ppm DDT for 3 weeks (unspecified isomeric composition) (Braham and Neal 1974).

Amphibians. Body weight loss was significantly exposure-related in tadpoles of the common frog (*Rana temporaria*) exposed for 1 hour to 0.01–10 ppm *p,p'*-DDT in the water column (Cooke 1970a).

Birds. Body weights were not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) by daily oral administrations of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDE, and 15 mg *p,p'*-DDD for 10 weeks (Greichus et al. 1975); doses were injected into the first fish fed to the birds each day. American kestrels (*Falco sparverius*) that died after exposure to 2.8 ppm *p,p'*-DDE in the diet for 14–18 months lost between 30 and 35% of their body weight; kestrels that survived the chronic dietary exposure lost an average of 16% of their body weight (Porter and Wiemeyer 1972). Bald eagles (*Haliaetus leucocephalus*) experienced weight loss of up to 49% after 10–16 weeks on diets containing 10–4,000 ppm technical-grade DDT (Chura and Stewart 1967).

Body weights of mallard ducklings (*Anas platyrhyncus*) were not significantly affected by a 10-day oral exposure to *p,p'*-DDT at up to 2,000 ppm in the diet (Friend and Trainer 1974a), but in apparent

contradiction, body weight gain was "suppressed" in Mallard ducklings fed DDT (unspecified composition) at 500–900 ppm in the diet for 10 days (Friend and Trainer 1974b).

Body weights of adult female Japanese quail (*Coturnix coturnix japonica*), but not males, were significantly decreased after daily intramuscular injections of 5 mg *o,p'*-DDT for 4 days (Cooke 1970b); male body weights were also unaffected by nine oral administrations of 10 mg DDT over a 3-week period. In another study in Japanese quail, body weights were not significantly affected by dietary exposure to up to 100 ppm *p,p'*-DDE and *p,p'*-DDT for 21 days (Bunyan and Page 1973; Bunyan et al. 1972). Growth of Japanese quail as measured by body weight was comparable to control group growth throughout a 3-generation reproductive toxicity assay in which birds were administered up to 50 ppm DDT (unspecified isomeric composition) in the diet (Shellenberger 1978).

Bobwhite quail (*Colinus virginianus*) lost between 2 and 24% of body weight after 5 days of feeding on diets containing $400 ppm technical-grade DDT (Hill et al. 1971); wild quail lost appreciably more weight than farm-raised birds at the same exposure levels. No effect on hen body weight was seen in laying bobwhite quail orally administered up to 20 mg/bird of DDT (unspecified isomeric composition) by capsule every other day during a 4-week exposure period (Wilson et al. 1973).

Chicken body weights were not significantly affected by 6-week dietary exposure to 400 or 800 ppm (Glick 1974) or by #81 days of daily subcutaneous injections of DDT (isomeric composition unspecified) for a cumulative dose of 2–3 grams (Burlington and Lindeman 1952). However, a more recent study found a slight but significant loss of body weight, accompanied by a significant reduction in food consumption, in hens orally administered 40 mg/hen of technical-grade DDT for 5 days (Chen et al. 1994). No effect on body weight was observed in chicken hens fed *p,p'*-DDT at #200 ppm in the diet for 12 weeks (Davison and Sell 1972).

Pheasant (*Phasianus colchicus*) adult females fed diets containing 400 ppm DDT (unspecified isomeric composition) for at least 10 weeks lost weight, but females fed 100 ppm DDT gained weight (Genelly and Rudd 1956).

Nonsignificant body weight loss was seen in ringed turtle doves (*Streptopelia risoria*) fed *p,p'*-DDE at 10 or 50 ppm in the diet for 63 days (Haegele and Hudson 1977). No significant change in body weight was seen in ringed turtle doves fed diets containing up to 200 ppm DDE (unspecified isomeric composition) for 8 weeks (Heinz et al. 1980). Homing pigeons (*Columba livia*) that died after several weeks of oral exposure to *p,p'*-DDE at $36 mg/kg/day by capsule showed an average weight loss of approximately 33% of their original weights, and birds that survived 8 weeks of exposure had lost an average of 2% of body weight; controls showed no change in body weight (Jefferies and French 1972).

House sparrows (*Passer domesticus*) fed diets containing 320–700 ppm technical-grade DDT for 5 days lost between 10 and 12% of their body weight on the average (Hill et al. 1971). Body fat content and body weight gain were significantly decreased in an exposure-related manner in white-throated sparrows (*Zonotrichia albicollis*) fed diets containing 5–25 ppm technical-grade DDT for 31 days prior to the beginning of the migratory season (Mahoney 1975); birds fed *p,p'*-DDE at 5 or 25 ppm in the diet for 6 weeks showed no significant alteration in body weight gain or body fat indices. No effect on body weight was seen in redstarts (*Phoenicurus phoenicurus*) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974).

Metabolic Effects. No experimental studies were located regarding metabolic effects in reptiles or amphibians from exposure to DDT/DDE/DDD. Metabolic rate was increased in mammals and birds receiving relatively low oral bolus (capsular) doses of DDT, and decreased at relatively high bolus doses. Blood calcium was consistently unaffected by DDT/DDE/DDD treatment in birds after dietary or parenteral exposures; no effect on blood calcium was seen in seven studies using six bird species after acute or intermediate durations. Altered blood calcium was seen only after relatively high, intermediate duration oral bolus (capsular) exposure in one bird species. Two studies in birds reported increased blood pCO_2 after acute oral exposures, although the significance of this is uncertain.

Mammals. Metabolic rate as measured by O_2 consumption rate was significantly increased compared to controls in short-tailed shrews (*Blarina brevicauda*) fed an earthworm diet containing an average of 16.6 ppm DDT (unspecified isomeric concentration) for 1 week, but not after 2 or 3 weeks of exposure; treated shrews that were starved after exposure also showed an increase in metabolic rate compared to a control group that showed reduced metabolic rate in response to starvation (Braham and Neal 1974).

Birds. Bobwhite quail (*Colinus virginianus*) fed on diets containing 10–150 ppm technical-grade DDT for at least 6 weeks showed increased (not statistically significant) metabolic rate compared to controls as measured by oxygen consumption at all ambient temperature levels tested between 5 and 40 EC, except at thermal neutrality at 30 EC, at which oxygen consumption was comparable to controls (Lustick et al. 1972; Peterle et al. 1973). In low-temperature stressed quail, shivering occurred in many birds but was most pronounced in birds that died after being fed DDT, while at the highest temperatures tested, several DDT-treated birds collapsed while none of the controls collapsed; the authors surmised that dietary DDT may have reduced the upper critical temperature of the thermoneutral zone from 40 to 35 EC (Lustick et al. 1972; Peterle et al. 1973). It is possible that the increased severity of "shivering" observed in stressed quail that eventually died may actually have been DDT-induced neurotoxicity (i.e., tremors), since similar responses that have been attributed to neurotoxicity have been observed in several other studies of energy-stressed birds (Appendix D.4, Neurological/Behavioral effects). After 11 weeks consuming *p,p'*-DDT in capsules at 3 mg/kg/day, homing pigeons (*Columba livia*) showed significantly increased oxygen consumption (with a significant increasing time-trend), but at 36 mg/kg/day for 11 weeks, there was a significant decrease in oxygen consumption (with a significant decreasing time trend) (Jefferies and French 1971). Jefferies and French (1971) found that body temperature significantly decreased over time in homing pigeons in response to daily oral exposure to *p,p'*-DDT in capsules at 36 mg/kg/day or higher for up to 8 weeks.

Blood calcium was not significantly affected in double-crested cormorants (*Phalacrocorax auritus*) fed diets containing #25 ppm total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Serum calcium was not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) exposed by daily oral administrations of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDE, and 15 mg *p,p'*-DDD for 10 weeks (Greichus et al. 1975); doses were injected into the first fish fed to the birds each day. Blood calcium levels during egg laying was not significantly affected in Pekin ducks (domesticated Mallards; *Anas platyrhyncus*) fed diets containing 250 ppm DDE (unspecified isomeric composition) for 10 days; no other exposure levels were tested (Peakall et al. 1975). Decreased serum calcium was seen in laying bobwhite quail (*Colinus virginianus*) administered 20 mg/bird of DDT (unspecified isomeric composition) by capsule every other day during a 4-week exposure period, but not at 10 mg DDT/bird (Wilson et al. 1973). Serum calcium was not significantly affected in Japanese quail (*Coturnix coturnix japonica*) exposed by four consecutive daily intramuscular injections with 5 mg *o,p'*-DDT or by nine oral administrations of 10 mg/bird of *o,p'*-DDT over 3 weeks (Cooke 1970b). No effect on plasma calcium levels was seen in cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days starting on the 8th day posthatch; daily dose was unreported, but cumulative dose was 2–3 g/cockerel (Burlington and Lindeman 1952). No significant effect on plasma calcium was seen in hens fed 40 mg/hen of technical DDT for 5 days (Chen et al. 1994). Blood calcium

during egg laying was not significantly affected in ringed turtle doves (*Streptopelia risoria*) by a 3-week dietary exposure to 100 ppm DDE (unspecified isomeric composition); no other exposure levels were tested (Peakall et al. 1975).

Blood pCO_2 (partial pressure of CO_2 in blood in units of mm Hg) was significantly increased in adult male Japanese quail (*Coturnix coturnix japonica*) fed diets containing $10 ppm technical-grade DDT (but not at 3 ppm) for 2 weeks, but at 6 weeks, there was a significant increase only at 10 ppm and not at higher exposure levels; there was no consistent effect in females (Kenney et al. 1972). Significantly increased blood pCO_2, but no significant effect on pO_2 and blood pH, was also seen in crowned guinea fowl (*Numida meleagris*) compared to pre-exposure levels after a 5-day oral gavage exposure to approximately 75 mg/kg/day technical-grade DDT (Fourie and Hattingh 1979).

Significantly depressed serum potassium levels were observed in white pelicans (*Pelecanus erythrorhynchos*) administered daily oral doses of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE for 10 weeks (Greichus et al. 1975); no significant effects were seen in serum inorganic phosphorus, uric acid, magnesium, or sodium. Blood sodium, urea nitrogen, and phosphorus were not significantly affected in double-crested cormorants fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks (Greichus and Hannon 1973). Plasma osmolality and plasma sodium were significantly increased in white Pekin ducks fed 100 ppm DDE in the diet for a total of 15 days; plasma potassium was not significantly affected (Miller et al. 1976). Crowned guinea fowl showed significantly decreased serum potassium and urea nitrogen compared to pre-exposure levels after a 5-day gavage exposure to approximately 75 mg/kg/day technical-grade DDT (Fourie and Hattingh 1979); no significant effect was seen on blood sodium or osmolarity. No effect on plasma phosphorous level was seen in cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days starting on the 8th day posthatch; daily dose was unreported, but cumulative dose was 2–3 g/chicken (Burlington and Lindeman 1952). Significantly decreased plasma sodium was seen in ringed turtle doves fed diets containing 200 ppm (but not at #20 ppm) of DDE (unspecified isomeric composition) for 8 weeks; no significant effect on plasma potassium was seen (Heinz et al. 1980).

Other Systemic Effects. No experimental studies were located regarding other systemic effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD.

Birds. Nasal gland ("salt gland") secretion rate was not significantly affected in white Pekin ducks (domesticated Mallards; *Anas platyrhyncus*) fed 50 ppm DDE (unspecified isomeric composition) in the diet for a total of 7 days (Miller et al. 1976). In Mallards maintained on fresh water (but not in those maintained on saltwater), nasal gland secretion rate was significantly depressed when challenged with intravenous saltwater injections after up to 9 days of feeding on diets containing $10 ppm DDE (unspecified isomeric composition) (Friend et al. 1973).

No significant changes compared to controls were seen in lactic, malic, α-glycero-phosphate, or glucose-6-phosphate dehydrogenase activities in extracts of homogenated liver, brain, breast muscle, and kidney tissue from redstarts (*Phoenicurus phoenicurus*) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974).

D.3 Immunological and Lymphoreticular Effects

No experimental studies were located regarding immunological or lymphoreticular effects in wild mammals, reptiles, or amphibians from exposure to DDT/DDE/DDD. Spleen weights were unaffected in several bird species. Immunological function in chickens was impaired after dietary exposure to DDT, but not in Mallards after similar exposures.

Birds. Spleen weight was not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) administered a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE per day for 10 weeks (Greichus et al. 1975). Double-crested cormorants (*Phalacrocorax auritus*) fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks showed no significant change in spleen weight (Greichus and Hannon 1973). Spleen and bursa weights were not significantly affected in chickens fed diets containing 500 ppm of *p,p'*-DDT for 5 weeks (Glick 1974).

Serum immunoglobulin levels were not significantly affected in white pelicans exposed by daily oral administrations of a combination of 20 mg *p,p'*-DDT, 15 mg *p,p'*-DDD, and 15 mg *p,p'*-DDE for 10 weeks (Greichus et al. 1975). The antibody titre in response to bovine serum albumin (BSA) injections was significantly decreased in domestic fowl chicks fed diets containing 400 ppm of *p,p'*-DDT (but not at 200 ppm) for 5 weeks and then had food withheld for 4 days prior to injection with BSA (but not in birds that did not have food withheld); serum IgG and IgM were depressed at exposure levels of $200 ppm for 4 weeks (Glick 1974). Plaque formation in response to sheep red blood cell challenge and an index of phagocytic activity were not significantly changed relative to controls in domestic fowl chicks fed diets containing 500 ppm of *p,p'*-DDT for 5 weeks (Glick 1974). A 10-day dietary exposure to up to 900 ppm of *p,p'*-DDT in the feed did not significantly affect hepatitis-induced mortality or incidence liver lesions in Mallard ducks (*Anas platyrhyncus*) (Friend and Trainer 1974b).

"Severe dissolution" of bursal follicles and vacuolation with loss of medullary cells was observed in the bursae of domestic fowl chicks fed diets containing 500 ppm of *p,p'*-DDT for 5 weeks (Glick 1974).

D.4 Neurological/Behavioral Effects

No experimental studies were located regarding neurological or behavioral effects in reptiles from exposure to DDT/DDE/DDD.

Neurological effects (tremors, convulsions, hyperactivity, and behavioral changes) were observed in mammalian wildlife, amphibians, and birds experimentally exposed to DDT or DDE, particularly after administration of lethal doses or after administration of lower doses when food intake was restricted. The most commonly reported neurological effect was tremors. Studies generally did not offer explanations as to the possible mechanisms that caused tremors, although it is not unreasonable to assume a mechanism similar to that seen in laboratory animals (see Section 3.6.2, Mechanisms of Toxicity). Diets were experimentally restricted in several studies to simulate the health effects of DDT/DDE/DDD mobilized from fat during periods of energetic stress in the wild such as may occur, for example, during periods of nesting, migration, or thermal or other stress. Reviews (EPA 1975; WHO 1989) have postulated that during periods of energy stress, DDT mobilized from fat is redistributed to the brain (presumably because of the high lipid content in brain tissue) where it induces neurological effects and death. A study in bats (Clark and Kroll 1977) demonstrated that DDT residues in the brain increase substantially when the diet was restricted. Although a direct action on the central nervous system in wildlife has not been confirmed by observations of brain lesions, one study in birds did show significant decreases in brain neurotransmitter levels that were associated with increased brain DDE residue levels after sublethal dietary exposures (Heinz et al. 1980). Alterations in neurotransmitter levels may explain changes in bird behavior that were observed in several species. However, most available data suggest that wildlife species may not be sensitive sentinels of neurological effects in humans because the most prominent neurological effects in wildlife occurred primarily at lethal exposure levels or in energy-stressed animals at lower exposure levels.

Mammals. Tremors, convulsions, and hyperactivity ("running fits") were observed in 16 of 40 short-tailed shrews (*Blarina brevicauda*) that died after consuming *p,p'*-DDT in the diet *ad libitum* for 14 days at unreported concentrations (the range of concentrations was sufficient to identify an LC_{50} of 1,784 ppm)

(Blus 1978). Prolonged tremors were seen in free-tailed bats (*Tadarida brasiliensis*) fed diets containing 107 ppm DDE (unspecified isomeric composition) for 40 days and subsequently starved; tremors were observed only during starvation in treated bats but not in control bats (Clark and Kroll 1977). Tremors were seen in big brown bats (*Eptesicus fuscus*) within 2.5 hours of a single oral dose of technical-grade DDT at 800 mg/kg, and were seen up to 14 days postdosing in virtually all bats administered DDT at doses ranging from 20 to 800 mg/kg (Luckens and Davis 1964). Pipistrelle bats also showed tremors the day before dying from a single oral exposure to >45 mg/kg of *p,p'*-DDT (Jefferies 1972).

Amphibians. Common frog (*Rana temporaria*) tadpoles showed uncoordinated movement and signs of hyperactivity after 1 hour of exposure to as low as 0.1 ppm *p,p'*-DDT (but not at 0.01 ppm) (Cooke 1970b), and transient uncoordinated hyperactivity when exposed to 0.001 ppm *p,p'*-DDT for as few as 5 days (Cooke 1973a). Similar neurological symptoms were seen in common frog tadpoles exposed as eggs to DDT for only 24–48 hours (Cooke 1972). Tadpoles of common frogs, common toads (*Bufo bufo*), and smooth newts (*Triturus vulgaris*) showed hyperactivity and abnormal movement when exposed to DDT (unspecified isomeric composition) for 24–48 hours during metamorphosis (Cooke 1972). Adult common frogs showed hyperactivity, tremors, lack of muscular coordination, and weakness several days after a single lethal oral dose of DDT (isomer not reported) at an unreported dose level; adult frogs who did not die within 10 days of the exposure recovered fully, and did not show further signs of neurotoxicity (Harri et al. 1979). Adult common frogs administered DDT (unspecified isomeric composition) in 16 twice-weekly oral doses of 0.6 mg/kg/dose in gelatin capsules showed similar signs of neurotoxicity and eventually died, but only when food was withheld at the time of exposure; frogs that were fed at the time of dosing did not show any signs of toxicity (Harri et al. 1979).

Birds. Brain weight was not significantly affected in white pelicans (*Pelecanus erythrorhynchos*) that consumed a diet of fish containing an unreported dose of a mixture of *p,p'*-DDT (40%), *p,p'*-DDE (30%), and *p,p'*-DDD (30%) for 10 weeks, and then subjected to a restricted diet for 2 weeks (Greichus et al. 1975). Double-crested cormorants (*Phalacrocorax auritus*) fed diets containing up to 25 ppm of total DDT/DDE/DDD for 9 weeks showed no significant change in brain weight (Greichus and Hannon 1973). No effect on brain weights was seen in redstarts (*Phoenicurus phoenicurus*) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974).

No gross brain lesions were observed in 6-week-old pheasant chicks (*Phasianus colchicus*) fed 100 ppm technical-grade DDT for up to 101 days or 500 ppm for up to 23 days (Azevedo et al. 1965).

Brain dopamine levels were significantly decreased in ringed turtle doves after 8-week dietary exposures of 20 and 200 ppm DDE (unspecified isomeric composition), but not when fed 2 ppm, and brain norepinephrine was significantly decreased at 200 ppm, but not at 2 or 20 ppm (Heinz et al. 1980). A significant negative correlation was seen between neurotransmitter levels and DDE residues in the brain (Heinz et al. 1980).

Bald eagles (*Haliaetus leucocephalus*) showed tremors before dying from consuming diets containing technical-grade DDT at 160 ppm or greater for 15 to 112 days, but tremors were not observed in eagles that did not die after consuming 10 ppm diets for up 120 days (Chura and Stewart 1967). Tremors were also seen in two out of three bald eagles before dying after administration of 4,000 ppm technical-grade DDT in the diet for at least 15 days (Locke et al. 1966). An adult and an immature male American kestrel (*Falco sparverius*) fed diets containing 2.8 ppm *p,p'*-DDE exhibited tremors prior to death during exposures that ranged from 14 to 16 months in duration (Porter and Wiemeyer 1972). Double-crested cormorants (*Phalacrocorax auritus*) fed diets containing #25 ppm total DDT/DDE/DDD for 9 weeks showed tremors and convulsions prior to death (Greichus and Hannon 1973).

Pheasants exhibited trembling before death from consuming 100 ppm technical-grade DDT in the diet for at least 22 days (Azevedo et al. 1965). Bobwhite quail (*Colinus virginianus*) showed "slight" tremors during a 5-day dietary exposure to 800 ppm technical-grade DDT in the feed; more severe neurotoxic signs, including convulsions, were seen in quail fed 1,600 ppm (Hill et al. 1971). Premortality tremors were seen in Japanese quail fed diets containing $700 ppm (lowest level tested) of *p,p'*-DDT for up to 20 days (Gish and Chura 1970). Japanese quail (*Coturnix coturnix japonica*) provided with an earthworm diet containing 298 ppm DDT (unspecified isomeric composition) for up to 5 days exhibited tremors before dying (Boykins 1967), but showed no tremors or convulsions after consuming #100 ppm *p,p'*-DDE in a dry diet of mash for 12 weeks (Dieter 1974). Pharol D-1 quail fed 40 ppm *p,p'*-DDT for 12 weeks showed tremors, but did not die (Davison et al. 1976).

House sparrows, cardinals (*Richmondena cardinalis*), and blue jays (*Cyanocitta cristata*) each showed tremors after consuming technical-grade DDT at concentrations in the diet ranging from 320 to 910 ppm for 5 days (Hill et al. 1971). Tremors were seen in house sparrows (*Passer domesticus*) provided with drinking water containing 12% DDT (unspecified isomeric composition) or earthworm diets containing at least 86 ppm DDT for 6 or fewer days (Boykins 1967). "Severe" tremors were observed prior to the death of cowbirds (*Molothrus ater*) fed diets containing 500 ppm of *p,p'*-DDT for at most 12 days (Stickel et al. 1966). Trembling accompanied by significantly decreased body temperature and oxygen consumption was seen in homing pigeons (*Columba livia*) orally administered *p,p'*-DDT at 36 mg/kg/day for 12 weeks and then 54 mg/kg/day for 6 weeks (Jefferies and French 1971). Trembling was observed in pigeons that died after oral exposure to *p,p'*-DDE at $36 mg/kg/day by capsule for up to 56 days (Jefferies and French 1972).

Ataxia was observed in 8 chickens fed diets containing 1,600 ppm of *p,p'*-DDT for <2 to 4 weeks before dying, but no effects were seen at 800 ppm (Glick 1974). Uncoordinated movement was seen in pheasants that died after consuming 100 ppm technical-grade DDT in the diet for at least 22 days (Azevedo et al. 1965). Bobwhite quail (*Colinus virginianus*) showed "slight" balance disturbances during a 5-day dietary exposure to $800 ppm technical-grade DDT (Hill et al. 1971). Balance disturbances were seen in house sparrows, cardinals (*Richmondena cardinalis*), and blue jays (*Cyanocitta cristata*) after consuming technical-grade DDT at concentrations in the diet ranging from 320 to 910 ppm for 5 days (Hill et al. 1971).

Predatory response time and attack rate were not significantly affected in American kestrels fed diets containing 6 ppm *p,p'*-DDE for 4 months, both in comparison to pretreatment rates and compared to controls (Rudolph et al. 1983). Mallard peck order was apparently not affected by consuming 2.5–250 ppm technical-grade DDT in the diet for at least 29 days (Peterle et al. 1973). The onset of nocturnal restlessness indicative of normal migratory behavior (Zugunruhe) appeared to be delayed for 1 week in white-throated sparrows (*Zonotrichia albicollis*) fed 5–25 ppm technical-grade DDT in the diet for 31 days prior to the migratory season and for approximately 2 weeks during the initial phases of the migratory season (followed by a 14-week observation period on untreated diet); the behavior was abnormally increased toward the end of the migratory season after exposure had ceased (Mahoney 1975). A similar late migratory season increase in nocturnal restlessness was seen in white-throated sparrows fed 5–25 ppm DDE (unspecified isomeric composition) in the diet for 31 days prior to the migratory season and for approximately 2 weeks during the initial phases of the migratory season (Mahony 1975). Significantly decreased courting behavior was seen in ringed turtle doves fed *p,p'*-DDE at 10 or 50 ppm in the diet for 63 days (Haegele and Hudson 1977). Courtship behavior in ringed turtle doves (*Streptopelia risoria*) was not significantly affected by a 3-week pre-pairing dietary exposure to 100 ppm *p,p'*-DDE, but when birds had also been on a 10% food reduction, courtship behavior was "practically eliminated"; a significant interaction between DDE exposure and restricted diet was apparent, because the reduction in courting behavior was not as pronounced in birds on the restricted diet that had not been exposed previously to DDE (Keith and Mitchell 1993). Decreased nest attendance by parental birds was

reported in ringed turtle doves fed diets *ad libitum* containing 100 ppm *p,p'*-DDE for a 3-week period prior to pairing for mating (Keith and Mitchell 1993); decrease in chick survival appeared to be related to nest attendance, and the authors hypothesized that losses in young birds were related to decreased food intake by the chicks. No significant effect on locomotor activity pattern was reported in redstarts (*Phoenicurus phoenicurus*) administered a cumulative oral dose of 126 µg *p,p'*-DDT administered in equal daily doses over a 12-day period (Karlsson et al. 1974).

D.5 Reproductive Effects

Little information is available concerning reproductive effects in reptiles, amphibians, and wild mammals from exposure to DDT/DDE/DDD. In birds, the well-publicized decline in wild raptor populations, including the bald eagle, during the 1950s and 1960s was attributed partly to reproductive impairment, particularly eggshell thinning (see Appendix D.6, Eggshell Thinning in Birds). Egg production, fertility, and hatchability were largely unaffected in numerous studies in a variety of bird species. However, increased embryolethality, decreased egg size, delayed oviposition after mating, and increased testicular effects were observed with some regularity among experimental studies in birds. Several authors speculated that the effects were due to DDT-induced hormonal imbalances, and in fact, blood hormone levels (estrogen, luteinizing hormone) were altered in three of four studies in birds consuming either DDT or DDE in the diet. While the mechanisms of toxicity for these effects have not been thoroughly investigated in wildlife, and thus the direct relevance to human health is uncertain, the consistency of certain reproductive effects suggests that wildlife species may be appropriate sentinels for reproductive toxicity of DDT/DDE/DDD in humans.

Mammals. Short-tailed shrews showed testis weights that were not significantly different from controls after consuming an earthworm diet containing an average of 16.6 ppm DDT for 3 weeks (unspecified isomeric composition) (Braham and Neal 1974). Similar to the associations made between DDT and preterm deliveries in humans (Saxena et al. 1980, 1981; Wassermann et al. 1982), premature births in California sea lions (*Zalophus californianus californianus*) are associated with elevated DDT concentrations (DeLong et al. 1973). Mean blubber DDT in sea lions delivering preterm was 824.4 ppm, whereas blubber DDT in full term individuals averaged 103.2 ppm. However, the effect of preterm delivery could not be causally isolated to DDT, as PCBs were also significantly elevated in individuals having preterm deliveries.

Reptiles. Organochlorine contaminants, in general, and *p,p*'-DDE specifically, are thought to influence sexual dimorphism in the common snapping turtle (*Chelydra serpentina*) (de Solla et al. 1998). In normal snapping turtles, the cloaca of the female is located very close to the plastron when compared to males. However, snapping turtles in Ontario, Canada, with high *p,p*'-DDE concentrations (mean concentrations of 10.1 ppm at one site and 21.7 ppm at another site) lacked such sexual dimorphism in the distance between cloaca and plastron. De Solla et al. (1998) attributed this lack of sexual dimorphism to the antiandrogenic effects of *p,p*'-DDE.

Birds. Ovary weight and oviduct weight were not significantly affected in adult female Japanese quail (*Coturnix coturnix japonica*) administered *o,p'*-DDT by intramuscular injection at 5 mg/bird on each of 4 consecutive days (Cooke 1970b). Ovary weights were significantly reduced in an exposure-related manner in Japanese quail fed diets containing between 700 and 1,600 ppm *p,p'*-DDT for 20 days (Gish and Chura 1970); the effect was more pronounced in treatment groups with relatively lower body weights. Increases in oviduct weight and uterine glycogen content were observed in chickens and Japanese quail administered 50 mg/bird of *o,p'*-DDT (but not in birds administered *p,p'*-DDT) by intraperitoneal injection on 3 consecutive days; the increases were comparable to those seen in birds injected 3 times with 0.5 mg 17β-estradiol (Bitman et al. 1968). Ovary and oviduct weights were

significantly decreased in ringed turtle doves fed diets containing 100 ppm *p,p'*-DDE for a 3-week period prior to pairing for mating (Keith and Mitchell 1993).

Latency time between first pairing and first oviposition was significantly increased compared to controls in Mallard hens fed diets containing 10 ppm DDE (isomeric composition not reported) for 2 months prior to pairing and through the breeding season (Vangilder and Peterle 1980). Delayed oviposition after initial pairing (compared to controls), attributed by the authors to delayed ovulation, was observed in Japanese quail fed diets containing either 100 ppm *p,p'*-DDT or 100 ppm *p,p'*-DDE (the only exposure levels tested) for up to 74 days (Cecil et al. 1971). The incidence of delayed oviposition was significantly increased in a dose-related manner in Bengalese finches (*Lonchura striata*) after dietary exposure to *p,p'*-DDT at up to 0.3 mg/bird/day (dose levels were not reported) for at least 15 weeks (Jefferies 1967, 1969). Jefferies (1967) hypothesized that the proximal cause of the delay in oviposition in Bengalese finches was delayed ovulation because of underdeveloped ovaries; he further postulated that DDT may have induced an estrogen-like inhibition of FSH and LH secretion by the pituitary thus, FSH and LH stimulation of ovary development may have been inhibited. An exposure-related significant increase (compared to controls) in median latency period between pairing for mating and the first oviposition was seen in ringed turtle doves (*Streptopelia risoria*) fed diets containing 10 and 40 ppm *p,p'*-DDE for approximately 90 days (Richie and Peterle 1979); in doves fed 40 ppm, there was also a delay and a nonsignificant suppression in peak serum LH levels after pairing, compared to controls (Richie and Peterle 1979), although the authors did not believe the findings established a clear causal relationship. Significantly delayed oviposition, associated with significantly reduced blood estradiol (compared to controls) and significantly increased hepatic enzyme metabolism of estradiol at 8 days postmating (but not in birds after completing their clutch), was observed in ringed turtle doves fed diets containing 10 ppm *p,p'*-DDT for 3 weeks prior to mating (Peakall 1970).

Egg production was not significantly affected in American kestrels (*Falco sparverius*) fed diets containing unreported levels (reportedly calculated to be "just short" of the lethal level) of DDT (unspecified isomeric composition) for at least a full year that encompassed two breeding seasons, nor was an effect seen in first-year young that were fed DDT at the parental dietary levels and bred in the second breeding season of the study (Porter and Wiemeyer 1969). Interestingly, mean egg production was significantly increased in two consecutive breeding seasons in barn owls fed diets containing 3 ppm DDE (unspecified isomer) for several months prior to the first breeding season and for almost 2 years before the second (Mendenhall et al. 1983). The reasons for this were compensatory egg production that occurred in the DDE-fed owls, which replaced eggs that were lost due to breakage, and re-nesting that occurred among owls that lost their entire clutches.

Egg production per hen was not significantly affected in mallards fed #40 ppm *p,p'*-DDT, *p,p'*-DDE, or technical-grade DDD in the diet either for several weeks or over 1 year (Heath et al. 1969). Egg production was not significantly affected in mallards (*Anas platyrhyncus*) exposed to 40 ppm *p,p'*-DDE in the diet for 1–4 months (Risebrough and Anderson 1975) or 10 ppm DDE (isomeric composition not reported) for 2 months prior to pairing and through the breeding season (Vangilder and Peterle 1980). Egg production and embryonation (the production of an embryo within the egg) were not significantly affected in black duck (*Anas rubripes*) clutches that were laid after approximately 5 months of exposure to 10 or 30 ppm (the only levels tested) *p,p'*-DDE in the diet (Longcore et al. 1971).

Egg production was significantly decreased in Japanese quail fed technical-grade DDT in the diet at 10–100 ppm (but not at 3 ppm) for up to 95 days (Kenney et al. 1972). However, egg production was not significantly affected in Japanese quail fed up to 200 ppm *p,p'*-DDE for 13 weeks or up to 40 ppm *p,p'*-DDT for 16 weeks, or in Pharol D-1 quail fed diets containing up to 40 ppm *p,p'*-DDT for 12 weeks (Davison et al. 1976). Egg production was significantly decreased in Japanese quail that were on restricted diets for approximately 10 days prior to dietary exposure to $700 ppm (lowest level tested) of

p,p'-DDT for 20 days, but not consistently in birds fed normal quantities of food prior to exposure (Gish and Chura 1970). Egg production was not significantly affected in chickens or in Japanese quail by dietary exposure to up to 100 ppm of DDT/DDE/DDD (a commercial mix of DDT compounds) for up to 10 weeks (Scott et al. 1975). There was no consistent effect on egg production in three consecutive generations of Japanese quail fed diets containing up to 50 ppm DDT (isomeric composition not specified) for their lifetime (Shellenberger 1978). Significantly reduced first-month egg production was observed in Japanese quail fed diets containing 100 ppm *p,p'*-DDE for up to 74 days, but not in those fed 100 ppm *p,p'*-DDT (the only exposure levels tested); egg production during 3 subsequent months was not significantly different from control levels in both treatment groups (Cecil et al. 1971). Chickens fed *p,p'*-DDT at up to 200 ppm in the diet for 12 weeks showed no significant changes relative to controls in egg production (eggs/bird; eggs/clutch) (Davison and Sell 1972). No significant effect on egg production was observed in chickens orally administered 40 mg technical-grade DDT/chicken on 5 days (Chen et al. 1994). No effect on egg production was seen in laying bobwhite quail (*Colinus virginianus*) orally administered up to 20 mg/bird of DDT (unspecified isomeric composition) by capsule every other day during a 4-week exposure period (Wilson et al. 1973). Pheasant (*Phasiannus colchicus*) egg production was not significantly affected by exposure to up to 500 ppm technical-grade DDT in the diet for at least 21 days prior to egg-laying until either the beginning of egg-laying or through egg-laying (Azevedo et al. 1965).

Egg production was significantly reduced by an average of 13.5% in ringed turtled doves fed diets containing 40 ppm of *p,p'*-DDE for 126 days (Haegele and Hudson 1973). Clutch size was unaffected in Bengalese finches by dietary exposure to *p,p'*-DDT at estimated dose levels of up to 0.3 mg/bird/day for at least 15 weeks (Jefferies 1969).

Egg weight and lipid content were reportedly not significantly affected compared with controls in barn owls (*Tyto alba*) fed 3 ppm DDE (isomeric composition not reported) in the diet for up to 2 years that encompassed two breeding seasons (Mendenhall et al. 1983). Egg weights were not significantly affected in chickens fed *p,p'*-DDT at up to 200 ppm in the diet for 12 weeks relative to controls (Davison and Sell 1972). Decreased mean egg weight was observed in eggs of bobwhite quail hens orally administered 20 mg/bird of DDT (unspecified isomeric composition) by capsule every other day during a 4-week exposure period, but not in birds administered 10 mg/bird (Wilson et al. 1973). Egg size was significantly decreased in a dose-related manner in Bengalese finches after dietary exposure to *p,p'*-DDT at up to 0.3 mg/bird/day (dose levels were not reported) for at least 15 weeks (Jefferies 1969). Significantly reduced mean egg weight was observed in ringed turtle doves (*Streptopelia risoria*) fed diets containing 10 ppm *p,p'*-DDT for 3 weeks prior to mating (Peakall 1970).

Egg fertility was not significantly affected in American kestrels fed diets containing unreported levels (reportedly calculated to be "just short" of the lethal level) of DDT (unspecified isomeric composition) for at least a full year that encompassed two breeding seasons, nor was an effect seen in first-year young that were fed DDT at the parental dietary levels and bred in the second breeding season of the study (Porter and Wiemeyer 1969). However, among parental groups of kestrels, significantly decreased egg hatchability was observed in at least one of the two breeding seasons (Porter and Wiemeyer 1969), and among yearling breeders, significantly decreased egg hatchability was observed in DDT-treated groups compared to untreated controls (Porter and Wiemeyer 1969). Egg hatchability was significantly decreased compared to controls in Mallard hens fed diets containing 10 ppm DDE (isomeric composition not reported) for 2 months prior to pairing and through the breeding season (Vangilder and Peterle 1980). Hatchability and fertility of pheasant eggs were not significantly affected by exposure to up to 500 ppm technical-grade DDT in the diet for at least 2 days prior to egg-laying until either the beginning of egg-laying or through egg-laying (Azevedo et al. 1965). Egg hatchability was not significantly affected in chickens or in Japanese quail exposed to #100 ppm of DDT/DDE/DDD (a commercial mix of DDT compounds) in the diet for up to 10 weeks (Scott et al. 1975). There was no consistent effect on egg

fertility or hatchability in three consecutive generations of Japanese quail fed diets containing up to 50 ppm DDT (isomeric composition not specified) for their lifetime (Shellenberger 1978). Egg hatchability was not significantly affected in Japanese quail fed 200 ppm technical-grade DDT in the diet for either the first or second week of the laying period (Jones and Summers 1968). Decreased fertility and hatchability were observed in eggs of bobwhite quail hens orally administered 20 mg/bird of DDT (unspecified isomeric composition) by capsule every other day during a 4-week exposure period, but not in birds administered 10 mg/bird (Wilson et al. 1973). Egg hatchability was reduced, but not significantly, in ringed turtled doves fed diets containing 40 ppm of *p,p'*-DDE for 126 days (Haegele and Hudson 1973).

Compared to controls, significantly increased embryolethality was observed in the eggs of yearling kestrels fed diets, since fledging, containing unreported levels of DDT (unspecified isomer) (Porter and Wiemeyer 1969). Embryonic survival was significantly decreased late in the incubation period in Mallard ducks fed $10 ppm *p,p'*-DDE for 2–3 weeks or for over 1 year; no significant effect on embryolethality was seen in ducks fed *p,p'*-DDT or technical-grade DDD for over 1 year (Heath et al. 1969). Embryolethality in intact eggs was significantly increased in black duck (*Anas rubripes*) clutches that were laid after approximately 5 months of exposure to 10 or 30 ppm (the only levels tested) *p,p'*-DDE in the diet (Longcore et al. 1971).

Overall reproductive performance score (a composite index accounting for egg production, egg hatchability, and fledging rate) was significantly decreased (primarily from a decrease in fledging rate, i.e., chick survival) compared with untreated controls in ringed turtle doves fed diets *ad libitum* containing 100 ppm *p,p'*-DDE for a 3-week period prior to pairing for mating; egg production was eliminated in birds that were fed DDE and were provided 10–30% less food than controls that were fed *ad libitum* (Keith and Mitchell 1993). See Appendix D.7, Developmental Effects, for further discussion of fledgling survival. In another study, although egg production, egg fertility, and egg hatchability in pheasants fed diets containing to up to 400 ppm DDT (unspecified isomeric composition) for at least 3 weeks were not significantly different from controls, the cumulative effect of the nonsignificant changes (reported as relative reproductive success rate-recruitment rate of chicks to 13 days of age) was markedly decreased in an exposure-related manner (Genelly and Rudd 1956).

Testis weight was not significantly affected in adult male Japanese quail orally administered 9 doses of 10 mg *o,p'*-DDT/bird over 3 weeks (Cooke 1970b). Testis weights were significantly reduced in an exposure-related manner in male Japanese quail fed diets containing between 700 and 1,600 ppm *p,p'*-DDT for 20 days (Gish and Chura 1970); the effect was more pronounced in treatment groups with relatively lower body weights. Testis size and secondary sex characteristics were markedly reduced compared to controls in White Leghorn cockerels injected subcutaneously for 60–89 days at DDT (unspecified isomer) dose levels that increased from 15 to 300 mg/kg/day during the exposure period (Burlington and Lindeman 1950). Testis weights and secondary sex characteristics were also "strikingly" decreased in cockerels orally administered (unspecified administration technique) DDT (unspecified isomer) at 6.25–50 mg/kg/day for 47 weeks (George and Sunararaj 1995); relative testis weight was decreased up to 20% of control testis weights. Testis weights were not significantly affected in Bengalese finches exposed to #0.3 mg/bird/day *p,p'*-DDT for at least 15 weeks (Jefferies 1969). Testis weights were unaffected in ringed turtle doves fed diets containing 100 ppm *p,p'*-DDE for a 3-week period prior to pairing for mating (Keith and Mitchell 1993).

No effect on spermatogenesis was observed in testes of bald eagles (*Haliaetus leucocephalus*) fed a diet containing 10 ppm technical-grade DDT for 60–120 days (Locke et al. 1966). Semen volume, percentage of live sperm, sperm motility, and sperm concentration were decreased and semen cholesterol concentration was increased in a dose-related manner in White Leghorn cockerels administered oral doses (unspecified administration technique) of DDT (unspecified isomeric composition) ranging from 6.25 to

50 mg/kg/day for 47 weeks (George and Sundararaj 1995); effects persisted for 21 weeks following cessation of exposure.

"Marked" testicular degeneration was seen in one of three bald eagles fed 4,000 ppm technical-grade DDT in the diet for 15 days; the other two birds died after 15 and 23 days of exposure (Locke et al. 1966). Testicular degeneration was seen in one eagle fed 160 ppm technical-grade DDT for 112 days, but not in another eagle that died after 76 days at the same exposure level (Locke et al. 1966). No gross gonad lesions were observed in 6-week-old chicks of pheasants fed 100 ppm technical-grade DDT for up to 101 days, or 500 ppm for up to 23 days (Azevedo et al. 1965). Testes of cockerels orally administered 50 mg/kg/day (but not at lower doses) of DDT (unspecified isomeric composition) for 47 weeks showed a variety of microscopic pathologies that persisted after cessation of exposure, most notably testicular atrophy, and including "markedly" irregular seminiferous tubule size and shape, desquamation of spermatogonia, and pyknosis of germ cells (George and Sundararaj 1995).

Plasma 17β-estradiol concentration was significantly decreased in chickens after a 5-day oral exposure to technical-grade DDT at 40 mg/hen/day (Chen et al. 1994). Blood estradiol was significantly reduced (compared to controls) at 8 days postmating in ringed turtle doves (*Streptopelia risoria*) fed diets containing 10 ppm *p,p'*-DDT for 3 weeks prior to mating, but not in birds allowed to complete their clutch (Peakall 1970). In ringed turtle doves fed 40 ppm *p,p'*-DDE for 90 days (but not in those fed 10 ppm), there was a delay and a nonsignificant suppression in peak serum LH levels after pairing for mating, compared to controls (Richie and Peterle 1979); a concurrent delay in oviposition was observed. Since LH is a glycoprotein gonadotropin that stimulates corpora lutea formation and ovulation in females (Hadley 1984), the delay and transient suppression of peak LH levels after mating could have caused the delay in oviposition by delaying ovulation, although Richie and Peterle (1979) did not believe that the evidence indicated a clear cause and effect association.

D.6 Eggshell Thinning in Birds

Eggshell thinning in birds reached widespread public awareness in the 1960s and 1970s largely because of field observations of eggshell thinning in high-profile raptors like the bald eagle, peregrine falcon, and osprey, and the association of these observations with abrupt population declines. Field observations and experimental studies established a scientific link between DDT/DDE/DDD exposure, particularly DDE, and avian eggshell thinning, which weighed significantly in the decision to ban most domestic crop uses of DDT in the 1970s (EPA 1975). A large body of literature was developed during the 1960s and 1970s regarding the effects of DDT/DDE/DDD on eggshell integrity in birds; experimental findings on eggshell thinning are provided below. In general, raptors, waterfowl, passerines, and nonpasserine ground birds were more susceptible to eggshell thinning than domestic fowl and other gallinaceous birds, and DDE appears to have been a more potent inducer of eggshell thinning than DDT (Cooke 1973b; EPA 1975; Lundholm 1997; WHO 1989). Further, reproductive disturbances associated with DDT/DDE/DDD exposure continue to be reported in North American populations of predatory birds and/or birds that migrate to regions such as South America where DDT is still used (Lundholm 1997).

There is some question, however, as to the relevance of avian eggshell thinning to human health. Birds possess a shell gland—a specialized segment of the oviduct—that has no anatomical or physiological counterpart in humans. The function of the shell gland is to lay down calcite ($CaCO_3$ – calcium carbonate) onto the developing avian egg to form the eggshell (EPA 1975). Reduced total calcium deposition as calcite, rather than reduced percent calcium in the eggshell, has been hypothesized to be the proximal cause of avian eggshell thinning (Davison 1978). Mechanisms of action that involve a direct action of DDT/DDE/DDD on the shell gland itself probably have no human relevance, but mechanisms of action that involve intermediate effects, such as reduced blood calcium, may have relevance to human health. Possible mechanisms of eggshell thinning in birds have been extensively studied and reviewed

(Cooke 1973b; EPA 1975; Lundholm 1997; Peakall et al. 1975; WHO 1989). Early experimental work focused the eggshell thinning mechanism debate on calcium metabolism as opposed to carbonate availability (EPA 1975).

An early review (Peakall et al. 1975) summarized two sets of hypotheses based on a discussion by Cooke (1973c) concerning possible mechanisms of reduced calcium deposition by the shell gland on the developing egg. One set of hypotheses (including effects to the thyroid, parathyroid, and adrenals, as well as hypotheses involving estrogen mimicry) suggested that the calcium supply to the eggshell gland was reduced, and involved an expected intermediate effect of decreased blood calcium. However, available data indicate that blood calcium level in birds was generally not sensitive to DDT/DDE/DDD exposures even in species in which eggshell thinning has been observed (Lundholm 1997; Peakall et al. 1975; see Appendix D.2, Systemic Effects, Metabolic, above). The alternative set of hypotheses suggested that the functionality of the shell gland itself in laying down the calcium-based shell was impaired in spite of an adequate supply of calcium. Shortly after the DDT ban, a leading hypothesis for DDE-induced eggshell thinning was an inhibition of calcium ATP-ase in the shell gland; calcium ATP-ase is believed to act as a pump to produce active transport of Ca^{+2} from blood in shell gland mucosal capillaries into the lumen of the shell gland (EPA 1975; Lundholm 1997). More recent work in ducks suggests that DDE does not directly inhibit calcium ATP-ase, but rather inhibits a signal for activating the calcium pump (Lundholm 1997). An estrogenic regulation of eggshell gland calcium secretion by DDT/DDE/DDD does not appear to be a tenable hypothesis (Lundholm 1997). The leading hypothesis for DDE-induced eggshell thinning involves an inhibition by *p,p'*-DDE (but not by *o,p'*-DDE or DDT or DDD isomers) of prostaglandin synthesis in the shell gland mucosa (Lundholm 1997). Lundholm (1997) postulated that shell gland prostaglandin E_2 acts as a signal for calcium ATP-ase transport of Ca^{+2} (coupled to bicarbonate transport) during eggshell formation, and summarized experimental work that demonstrated a direct inhibition of prostaglandin synthetase by *p,p'*-DDE.

There is still some question as to the primary mechanism of eggshell thinning, and reviewers have suggested that mechanisms of eggshell thinning may differ between bird species or differ with environmental conditions or physiological state for a given species. The following experimental results illustrate the potentially wide-spread occurrence of eggshell thinning in wild bird populations.

Barn owls (*Tyto alba*) showed a substantial increase in egg breakage during incubation associated with significantly thinned eggshells during two breeding seasons (60–82% breakage in 3 ppm DDE-fed owls vs. 3–5% in controls) encompassed within a 2-year exposure period; mean number of eggs hatched and mean number of young fledged per mated pair were significantly decreased in DDE-treated owls (isomeric composition of DDE not specified), but this was likely due to the increased egg fragility (Mendenhall et al. 1983). Among parental and yearling groups of breeding American kestrels, significantly increased egg disappearance (presumably from breakage or egg-eating by parental birds) and significantly decreased eggshell thickness were seen in at least one of two consecutive breeding seasons after kestrels were fed unreported levels of DDT (unspecified isomer) in the diet for at least 1 year that encompassed the two breeding seasons (Porter and Wiemeyer 1969).

Mallard ducks (*Anas platyrhynchus*) fed 25 ppm *p,p'*-DDT in the diet or $10 ppm *p,p'*-DDE for either 2–3 weeks or for over 1 year showed a significant increase in percent eggs cracked and a decrease in eggshell thickness; no eggshell effects were seen in ducks fed technical-grade DDD for over 1 year or in ducks fed #10 ppm DDT (Heath et al. 1969). Egg breakage, due to significantly decreased eggshell thickness, was increased compared to untreated controls in Mallards (*Anas platyrhyncus*) after dietary exposure to 40 ppm *p,p'*-DDE for 1–4 months (Risebrough and Anderson 1975). Eggshell thickness was significantly decreased compared to controls in Mallard hens fed diets containing 10 ppm DDE (isomeric composition not reported) for 2 months prior to pairing and through the breeding season (Vangilder and Peterle 1980). Percent of eggs that cracked during incubation and eggshell thinning were significantly

increased in black duck (*Anas rubripes*) clutches that were laid after approximately 5 months of exposure to 10 or 30 ppm (the only levels tested) *p,p'*-DDE in the diet (Longcore et al. 1971).

Japanese quail (*Coturnix coturnix japonica*) showed significantly decreased eggshell weight after being fed technical-grade DDT in the diet at $10 ppm for up to 95 days (Kenney et al. 1972). However, neither eggshell weight nor eggshell thickness were significantly affected in Japanese quail fed up to 200 ppm *p,p'*-DDE for 13 weeks or up to 40 ppm *p,p'*-DDT for 16 weeks, or in Pharol D-1 quail fed diets containing up to 40 ppm *p,p'*-DDT for 12 weeks (Davison et al. 1976). Breaking strengths of eggshells was not significantly affected in chickens or in Japanese quail by dietary exposure to up to 100 ppm of DDT/DDE/DDD (a commercial mix of DDT compounds) for up to 10 weeks (Scott et al. 1975). A nonsignificant increase in egg breakage and significantly decreased eggshell calcium were observed in Japanese quail fed 100 ppm *p,p'*-DDT for up to 74 days, but not in those fed 100 ppm *p,p'*-DDE; eggshell thickness and eggshell weight were not significantly affected in both exposure groups (Cecil et al. 1971). Chickens fed *p,p'*-DDT at up to 200 ppm in the diet for 12 weeks showed no significant changes relative to controls in eggshell weights, eggshell calcium, and eggshell thickness (Davison and Sell 1972). Decreased eggshell thickness and shell membrane thickness, along with decreased serum calcium, were observed in eggs of bobwhite quail hens (*Colinus virginianus*) orally administered 20 mg/bird of DDT (unspecified isomer) by capsule every other day during a 4-week exposure period; no changes were seen in birds administered 10 mg/bird every other day for 4 weeks (Wilson et al. 1973). No significant effect on eggshell thickness was seen in chickens orally administered daily doses of 40 mg technical-grade DDT/bird for 5 days (Chen et al. 1994).

Ringed turtle doves (*Streptopelia risoria*) fed diets containing 40 ppm *p,p'*-DDE for 126 days showed significantly reduced eggshell thickness by an average of 10% compared to controls (Haegele and Hudson 1973). Significantly reduced deposition of calcium in eggs (as measured by ^{45}Ca incorporation) was observed in ringed turtle doves fed diets containing 10 ppm *p,p'*-DDT for 3 weeks prior to mating; breeding females were orally administered ^{45}Ca on the day before pairing for mating (Peakall 1970). Peakall (1970) observed a significant decrease in eggshell weights associated with significantly reduced oviduct carbonic anhydrase (which is involved in the secretion of calcareous eggshell) activity in ringed turtle doves injected intraperitoneally with 150 mg/kg *p,p'*-DDE within 1 day of laying the first egg of the clutch; since exposure occurred at the end of egg development, the authors concluded that DDE interfered directly with eggshell formation at the oviduct, rather than indirectly by affecting blood estradiol. Eggshells were respectively 35 and 20% thinner than controls in ringed turtle doves after a 3-week dietary exposure to 100 ppm DDE (unspecified isomeric composition) and in Pekin ducks (domesticated Mallards: *Anas platyrhyncus*) fed diets containing 250 ppm DDE for 10 days; no other exposure levels were tested (Peakall et al. 1975).

D.7 Developmental Effects

No experimental studies were located regarding developmental effects in wild mammals from exposure to DDT/DDE/DDD.

Little information is available concerning developmental effects in amphibians, but effects in reptile and bird populations have received considerable attention. Studies of alligator populations at Lake Apopka in Florida, where a pesticide spill occurred in 1980, have reported various effects that may ultimately affect reproduction in the population, including reduced clutch viability (Woodward et al. 1993), altered steroidogenesis (Crain et al. 1997; Guillette et al. 1995), abnormal ovarian morphology and plasma 17β-estradiol levels in female alligators (Guillette et al. 1994), and reductions of phallus size and serum testosterone in male alligators (Guillette et al. 1994, 1995, 1996, 1997, 1999), compared to alligators at control lakes where environmental concentrations of pesticides including DDT/DDE/DDD were relatively low. The purported estrogenicity of DDT and other contaminants was hypothesized to have induced

hormonal imbalance in Lake Apopka alligators, causing the observed effects (Guillette and Crain 1996). Since the alligators were exposed to a complex mixture of environmental contaminants at Lake Apopka, the contribution of DDT/DDE/DDD to the observed effects is uncertain. However, findings in an experimental study of *in ovo* DDE exposures in alligators (Matter et al. 1998) support the hypothesis that certain DDT-related compounds induce estrogenic effects in reptiles, potentially ultimately affecting reproduction in the population. In general, reptiles may be particularly susceptible to the endocrine-altering effects of DDT/DDE/DDD, as many reptiles have environmental sex-determining mechanisms compared to the genetic sex-determining mechanisms in birds and mammals (Crain and Guillette 1998).

In birds, the most consistently reported developmental effect is a reduction in early posthatch survival in chicks after oral exposures to DDT or DDE in maternal birds. Reduced chick survival was observed in six bird species after acute to chronic experimental exposures, regardless of whether all of the maternal exposure occurred only prior to pairing for mating, only after mating (e.g., only during the hatching phase of reproduction), or both. While the mechanism of DDT-induced reduced chick survival has not been thoroughly studied, investigators have hypothesized that increased body burden of DDT in chicks may cause direct toxicity, or that reduction in parental care-giving among treated birds may result in chick malnutrition and poor survival. Other developmental effects in birds include a decreased ability to thermoregulate and behavioral alterations in chicks of treated parental birds, a reduced testicular development in chicks directly administered DDT, and development of ovarian tissue and oviducts in genetically male sea gulls exposed to DDT in the egg. Wildlife species may be appropriate sentinels of developmental effects in humans because certain effects, particularly reduced early survival in young, occurred consistently across several species under various exposure conditions.

Amphibians. Common frog (*Rana temporaria*) tadpole metamorphosis was significantly delayed compared to controls in tadpoles exposed for 28 days to 0.001 ppm *p,p'*-DDT in the surrounding water, but not in tadpoles exposed to 0.0001 ppm; abnormal spinal development was seen in 3/8 and 3/10 tadpoles in the low and high exposure groups, respectively, but not in controls (Cooke 1973a). Similar significantly delayed development was seen in common frog tadpoles exposed as eggs to 0.5 ppm DDT (unspecified isomeric composition) in the water column for only 24–48 hours (Cooke 1972). Altered facial features were observed in tadpoles of common frogs within 48 hours of a 1-hour exposure to $1 ppm *p,p'*-DDT; metamorphosis also appeared to be delayed (Cooke 1970a).

Reptiles. Recently, vitellogenin has been used as a biomarker for exposure to estrogenic contaminants (Folmar et al. 1996). Vitellogenin is a protein precursor of egg proteins that is produced in the liver of oviparous and ovoviviparous species and is normally found in the blood of these animals in measurable quantities; production of vitellogenin is stimulated by estrogens in the blood that are produced in the ovary; thus, it is normally absent in the blood of males (Hadley 1984). Production of vitellogenin (suggestive of estrogenic activity) was not induced in 21-day-old American alligators (*Alligator mississippiensis*) exposed *in ovo* during gonadal differentiation (at male-producing incubation temperatures) with *p,p'*-DDE and *o,p'*-DDE painted onto the outer surface of the eggshell at exposure levels ranging from 0.1 to 10 µg DDE/kg of egg (Matter et al. 1998). However, a significant decrease in percent male hatchlings and significantly decreased clitoral and penis sizes compared to controls were seen in 21-day-old alligators pooled from groups exposed *in ovo* to $1 µg *p,p'*-DDE/kg of egg painted onto the eggshell; no significant effects were seen in alligators pooled from groups exposed to #0.3 µg *p,p'*-DDE/kg of egg *in ovo* (Matter et al. 1998). Treatment of alligator eggs with *o,p'*-DDE during gonadal differentiation led to a significant decrease in percent male hatchlings in the low exposure group (#0.3 ppm), but not in the high exposure group ($1 ppm); nonsignificant decreases in clitoral/penis size were also seen in low exposure (but not in high-exposure animals) that were exposed *in ovo* to *o,p'*-DDE (Matter et al. 1998). The lack of a dose-response for these effects after *o,p'*-DDE exposures in this study warrants further investigation into the dose-response of gender alterations. Treatment (topical application) of eggs of the green sea turtle (*Chelonia mydas*) with *p,p'*-DDE in doses that resulted in up to

543 ng DDE/g egg (ppb) did not alter the sex ratio from what was expected based on temperature alone (Podreka et al. 1998). Furthermore, incubation time, hatching success, incidence of body deformities, hatching size, and weight were not significantly affected.

Birds. Significantly decreased early chick survival was observed compared to untreated controls among chicks of yearling kestrels fed unreported levels of DDT (unspecified isomer) in the diet since they were fledged the previous year (Porter and Wiemeyer 1969). Survival to 14 days posthatch was significantly decreased (approximately 35% decrease from control survival rate) in Mallard ducklings (*Anas platyrhynchus*) hatched from hens fed 25 ppm *p,p'*-DDT in the diet (but not at #10 ppm) for either 2–3 weeks or for over 1 year; no effect on posthatch survival was seen in ducklings of hens fed *p,p'*-DDE or technical-grade DDD for over 1 year (Heath et al. 1969). Duckling survival was significantly decreased in black duck (*Anas rubripes*) clutches that were laid after approximately 5 months of exposure to 10 or 30 ppm (the only levels tested) *p,p'*-DDE in the diet (Longcore et al. 1971). Pheasant chick (*Phasiannus colchicus*) survival was significantly decreased in an exposure-related manner in groups fed 100 (lowest level tested) and 400 ppm DDT (isomeric composition not reported) for at least 3 weeks (Genelly and Rudd 1956). Chick survival was significantly depressed during the first 7 days posthatch in pheasants fed diets containing 500 ppm technical-grade DDT (but not at 10–100 ppm) from at least 21 days prior to egg-laying until either the beginning of egg-laying or through egg-laying (Azevedo et al.1965). Survival of Japanese quail (*Coturnix coturnix japonica*) chicks was significantly reduced compared to controls in groups fed 200 ppm technical-grade DDT in the diet for either the first or second week of the laying period; egg hatchability was reportedly not significantly affected by DDT treatment, but 79% of chick mortality occurred within 3 days of hatching (Jones and Summers 1968); the authors concluded that lethal levels of DDT were transferred to the egg and accumulated in the developing chicks during incubation. Survival of Japanese quail, including chicks, was comparable to control group survival throughout a 3-generation reproductive toxicity assay in which birds were administered up to 50 ppm DDT (unspecified isomeric composition) in the diet (Shellenberger 1978). Fledging rate (i.e., chick survival) was decreased to 85% (compared to 100% in controls) in ringed turtle doves (*Streptopelia risoria*) fed diets *ad libitum* containing 100 ppm *p,p'*-DDE for a 3-week period prior to pairing for mating (Keith and Mitchell 1993); the decrease in chick survival was related to decreased nest attendance by parental birds, and the authors hypothesized that losses in young birds were related to decreased food intake by the chicks. No association between posthatch parental nest attendance and decreased survival in chicks was observed during the first 21 days posthatch in another study in ringed turtle doves at a lower exposure level. Survival during the first 21 days posthatch was significantly reduced compared to controls (by a factor of 2) in chicks of ringed turtle doves fed diets containing 40 ppm of *p,p'*-DDE for 126 days (Haegele and Hudson 1973).

Growth, as measured by body weight and foot pad length was reportedly not significantly affected in nestling red-tailed hawks (*Buteo jamaicensis*) fed technical-grade DDT at 20 mg/kg body weight every 4th day during an exposure period lasting up to 80 days (Seidensticker 1968). Growth of Japanese quail, as measured by body weight, was comparable to control group growth during a 3-generation reproductive toxicity assay in which birds were administered up to 50 ppm DDT (unspecified isomeric composition) in the diet throughout the study (Shellenberger 1978). No effect on chick weight was seen in bobwhite quail (*Colinus virginianus*) administered up to 20 mg/bird of DDT (unspecified isomeric composition) every other day during a 4-week exposure period (Wilson et al. 1973).

Failure to thermoregulate under cooling thermal stress (measured by cloacal temperature) was seen in a greater proportion (50%) of Mallard ducklings (*Anas platyrhyncus*) of hens fed diets containing 10 ppm DDE (unspecified isomeric composition) for 2 months prebreeding and through the breeding season than in ducklings of control hens (30%) (Vangilder and Peterle 1980). Among ducklings that did thermoregulate during cooling thermal stress, there was significantly greater body weight loss and

significantly decreased survival time compared to control ducklings that did thermoregulate (Vangilder and Peterle 1980).

Testis development was "markedly retarded" and development of secondary sex characteristics was inhibited in cockerels administered daily subcutaneous injections of DDT (unspecified isomeric composition) for up to 81 days starting on the 8th day posthatch; daily dose was unreported, but cumulative dose was 2–3 g/cockerel (Burlington and Lindeman 1952). Similar results were seen in another subcutaneous administration study in cockerels administered DDT (unspecified isomer) at dose levels that increased from 15 to 300 mg/kg/day over a 60- to 89-day exposure period (Burlington and Lindeman 1950).

Fry and Toone (1981) exposed sea gulls (*Larus californicus*), which are much more susceptible to the effects of estrogenic contaminants than domesticated chickens and quail, to different congeners of DDT and DDE. *o,p*'-DDT induced feminization (development of ovaries and oviduct) at all dosages tested (2–100 ppm) in genetically male gulls. *p,p*'-DDE induced such feminization only at high concentrations (20–100 ppm), whereas *p,p'*-DDT caused no abnormalities. Thus, the estrogenic action of *o,p*'-DDT has a gender-reversing effect in birds.

No effect on plumage development was seen in nestling red-tailed hawks (*Buteo jamaicensis*) fed technical-grade DDT at 20 mg/kg body weight every 4th day during an exposure period lasting up to 80 days (Seidensticker 1968).

Behavioral changes were observed in Mallard ducklings of hens that were fed 3 ppm *p,p'*-DDE in the diet for at least 3 months compared to controls; effects included significant hyper-responsiveness in a maternal approach stimulus test and significant under-responsiveness in a noise avoidance test (Heinz 1976).

D.8 Genotoxic Effects

No experimental studies were located regarding genotoxic effects in wildlife from exposures to DDT/DDE/DDD.

D.9 Cancer

No experimental studies were located regarding carcinogenic effects in wildlife from exposures to DDT/DDE/DDD.

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife

	Wild mammals, reptiles, and amphibians										
Adverse biological effect	Short-tailed shrew	Free-tailed bats	Big brown bat	Pipistrelle bat	Common frog	Bull frog	Common toad	Smooth newt	African clawed frog	Red-eared turtle	American alligator
Mortality											
immature					O_A^1						
mature	O_A^1	O_I^5	O_A^3	O_A^1	$O_{A,I}^4$	O_A^1					
Systemic Effects											
Respiratory											
Cardiovascular											
Gastrointestinal											
Hematological											
Musculoskeletal											
Hepatic											
liver weight	NO_I^4										
Renal											
Endocrine											
induction of vitellogenin in males									O_A^2	O_A^2	
Dermal/ocular											
Body weight											
loss or reduced gain	$NO_{A,I}^4$	O_I^7			O_A^1						
Metabolic											
O_2 consumption	O_A^4 NO_I^4										

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6 = o,p'-DDE;
7 = unspecified DDE;
8 = p,p'-DDD;
9 = o,p'-DDD;
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11 = unspecified DDD;
12 = DDT/DDE/DDD mixture

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Wild mammals, reptiles, and amphibians *(continued)*										
Adverse biological effect	Short-tailed shrew	Free-tailed bats	Big brown bat	Pipistrelle bat	Common frog	Bull frog	Common toad	Smooth newt	African clawed frog	Red-eared turtle	American alligator
Immunological/ Lymphoreticular											
Neurological/Behavioral											
tremors	O_A^1	O_I^7	O_A^3	O_A^1	$O_{A,I}^4$						
convulsions	O_A^1				$O_A^{1,4}$						
hyperactivity	O_A^1				O_I^4		O_A^4	O_A^4			
uncoordinated movement					$O_{A,I}^{1,4}$ O_I^4		O_A^4	O_A^4			
Reproductive											
testis weight	NO_I^4										
Developmental											
facial features					O_A^1						
spinal abnormalities					O_I^1						
hatchling sex ratio											O_A^5
penis size											O_A^5
clitoris size											O_A^5
induction of vitellogenin in males											$NO_A^{5,6}$
delayed development					O_I^1 O_A^4						
Genotoxic											
Cancer											

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9 = o,p'-DDD;
10 = technical grade DDD;
11 = unspecified DDD;
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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Birds: raptors, wading birds, water birds												
Adverse biological effect	Bald eagle	White pelican	American kestrel	Double-crested cormorant	Puffin	Mallard duck	Clapper Rail	Sandhill crane	White Pekin duck	Black duck	Barn owl	Red-tailed hawks	Gull
Mortality													
immature	O_I^3		O_C^5			$O_{A,I}^4$ $O_A^{1,5,10,11}$	O_A^1						
mature	O_I^3		O_C^5			$O_{A,I}^4$ O_A^1		O_A^1			NO_C^7		
Systemic Effects													
Respiratory													
Cardiovascular													
heart weight		NO_I^{12}		NO_I^{12}									
Gastrointestinal													
Hematological													
hematocrit				NO_I^{12}									
hemoglobin				NO_I^{12}									
Musculoskeletal													
Hepatic													
liver weight		O_I^{12}		O_I^{12}	NO_I^7								
vitamin A level		O_I^{12}		O_I^{12}									
microsomal MFO induction			O_C^7		O_I^7								
liver carotene level		NO_I^{12}		NO_I^{12}									
serum protein		O_I^{12}		NO_I^{12}									
Renal													
kidney weight		NO_I^{12}											

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8 = p,p'-DDD;
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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: raptors, wading birds, water birds *(continued)*

Adverse biological effect	Bald eagle	White pelican	American kestrel	Double-crested cormorant	Puffin	Mallard duck	Clapper Rail	Sandhill crane	White Pekin duck	Black duck	Barn owl	Red-tailed hawks	Gull
Endocrine													
adrenal weight						**NO$_I$**[3]							
adrenal cortico-medullary ratio						**NO$_I$**[3]							
thyroid weight						**O$_I$**[3]							
thyroid iodine uptake						**O$_I$**[3]							
plasma cortico-sterone level						**NO$_I$**[3]							
Dermal/ocular													
Body weight loss or reduced gain	**O$_I$**[3]	**NO$_I$**[12]	**O$_C$**[5]			**NO$_A$**[1] **O$_A$**[4]							
Metabolic													
blood urea		**NO$_I$**[12]		**NO$_I$**[12]									
blood K^+		**O$_I$**[12]							**NO$_I$**[7]				
blood Na^+		**NO$_I$**[12]		**NO$_I$**[12]					**O$_I$**[7]				
blood phosphorus		**NO$_I$**[12]		**NO$_I$**[12]									
blood Mg^{+2}		**NO$_I$**[12]											
blood Ca^{+2}		**NO$_I$**[12]		**NO$_I$**[12]					**NO$_A$**[7]				
plasma osmolality									**O$_I$**[7]				
Other													
nasal gland secretion rate						**O$_A$**[7]			**NO$_A$**[7]				

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: raptors, wading birds, water birds *(continued)*

Adverse biological effect	Bald eagle	White pelican	American kestrel	Double-crested cormorant	Puffin	Mallard duck	Clapper Rail	Sandhill crane	White Pekin duck	Black duck	Barn owl	Red-tailed hawks	Gull
Immunological/ Lymphoreticular													
hepatitis susceptibility						NO_A[1]							
spleen weight		NO_I[12]		NO_I[12]									
serum immuno-globulins		NO_I[12]											
Neurological/ Behavioral													
tremors	O_I[3]		O_C[5]	O_I[12]									
brain weight		NO_I[12]		NO_I[12]									
convulsions				O_I[12]									
predatory behavior			NO_I[5]										
social peck dominance						NO_I[3]							
Reproductive													
egg production			$NO_{I,C}$[4]			NO_C[1,3,5,10] NO_I[1,5,7,10]				NO_I[5]	$O_{I,C}$[7]		
egg size/weight											$NO_{I,C}$[7]		
embryo survival			$O_{I,C}$[4]			$NO_{I,C}$[1,10] $O_{I,C}$[5]				O_I[5]			
spermatogenesis	NO_I[3]												
testicular lesions	O_I[3]												
egg lipid content											$NO_{I,C}$[7]		
egg fertility			$NO_{I,C}$[4]										
timing of oviposition						O_I[7]							
egg hatchability			$O_{I,C}$[4]			O_I[7]							

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: raptors, wading birds, water birds *(continued)*

Adverse biological effect	Bald eagle	White pelican	American kestrel	Double-crested cormorant	Puffin	Mallard duck	Clapper Rail	Sandhill crane	White Pekin duck	Black duck	Barn owl	Red-tailed hawks	Gull
Eggshell Thinning													
egg breakage			$O_{I,C}$[4]			$O_{I,C}$[1,5] NO_C[10]				O_I[5]	$O_{I,C}$[7]		
eggshell thickness			$O_{I,C}$[4]			O_C[1,5] O_I[1,5,7] NO_C[10]			O_A[7]	O_I[5]	$O_{I,C}$[7]		
Developmental													
chick behavior						O_I[5]							
chick early survival			$O_{I,C}$[4]			$NO_{I,C}$[5,10] $O_{I,C}$[1] O_I[7]				O_I[5]			
nestling weight						O_I[7]						NO_I[3]	
nestling foot pad growth												NO_I[3]	
nestling plumage development												NO_I[3]	
chick thermo regulation						O_I[7]							
testicular feminization													O_I[2,12]
abnormal oviducts													O_I[2,12]
Genotoxic													
Cancer													

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6 = o,p'-DDE;
7 = unspecified DDE;
8 = p,p'-DDD;
9 = o,p'-DDD;
10 = technical grade DDD;
11 = unspecified DDD;
12 = DDT/DDE/DDD mixture

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Birds: gallinaceous birds						
Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Mortality							
immature	$O_I^{4,5}$ $O_A^{3,4,5,10}$	NO_C^{4} $O_A^{4,5,10}$	$O_A^{3,11}$				$O_I^{4,5}$ $O_A^{1,4,5,10,11}$
mature	$O_A^{3,4}$ $O_I^{4,5}$ NO_I^{4}	$O_A^{1,2}$ O_I^{1} NO_C^{4}					O_A^{4} $O_I^{3,4,5}$
Systemic Effects							
Respiratory							
gross lung lesions							NO_I^{3}
Cardiovascular							
gross heart lesions							NO_I^{3}
Gastrointestinal							
Hematological							
hemoglobin		NO_I^{5}			O_A^{3}	NO_I^{4}	
RBC count					O_A^{3}	O_I^{4} NO_I^{4}	
hematocrit	O_I^{3}	NO_I^{5}			O_A^{3}	NO_I^{4}	
Musculoskeletal							
gross muscle lesions							NO_I^{3}

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7 = unspecified DDE;
8 = p,p'-DDD;
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10 = technical grade DDD;
11 = unspecified DDD;
12 = DDT/DDE/DDD mixture

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: gallinaceous birds *(continued)*

Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Hepatic							
liver weight	O_I^3	$NO_I^{1,2,4,5,8}$ O_I^5				NO_I^1 NO_A^3	
plasma hepatic enzyme activity		O_I^5			O_A^3		
liver microsomal enzyme activity		$O_A^{1,2,5,6,8,9}$ $O_I^{1,4,5,8}$ NO_I^3				$O_{A,I}^3$	
microsomal protein level		NO_I^4				NO_A^3	
liver lipid	O_I^3						
liver microscopic lesions							NO_I^3
blood cholesterol					O_A^3	O_A^9	
serum lipid		$NO_{A,I}^2$					
serum protein					NO_A^3	NO_I^4	
fibrinogen level						NO_I^4	
prothrombin time						NO_I^4	
microsomal hormone metabolism	O_I^3					$O_{A,I}^3$	
Renal							
gross kidney lesions							NO_I^3
Endocrine							
adrenal weight	NO_I^3					NO_I^1	
adrenal microscopic lesions	O_I^3					O_A^9	
thyroid weight	O_I^3						
thyroid iodine uptake	O_I^3						
adrenal histology	O_I^3						
blood sugar level					O_A^3	NO_I^4	
Dermal/ocular							

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Birds: gallinaceous birds *(continued)*						
Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Body weight							
loss or reduced gain	NO_I^4 O_A^3	$NO_I^{1,2,5}$ O_A^2 NO_C^4				$NO_I^{1,4}$ O_A^3	O_I^4
decreased food consumption						O_A^3	
Metabolic							
oxygen consumption	NO_I^3						
blood urea					O_A^3		
blood K^+					O_A^3		
blood calcium	O_I^4	$NO_{A,I}^2$				NO_I^4 NO_A^3	
blood Na^+					NO_A^3		
blood lactic acid							
blood osmolarity					NO_A^3		
blood phosphorous						NO_I^4	
blood pCO_2		O_I^3			O_A^3		
blood pO_2					NO_A^3		
blood pH					NO_A^3		
Immunological/ Lymphoreticular							
spleen weight						NO_I^1	
bursa weight						NO_I^1	
bursal lesions						O_I^1	
BSA antibody formation						O_I^1	
SRBC plaque-forming cells						NO_I^1	
serum immunoglobulins						O_I^1	
phagocytic index						NO_I^1	

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: gallinaceous birds *(continued)*

Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Neurological/Behavioral							
tremors	O_A^3	NO_I^5 O_I^1 O_A^4		O_I^1			O_I^3
convulsions	O_A^3	NO_I^5					
ataxia						$O_{A,I}^1$	
loss of balance	O_A^3						
gross brain lesions							NO_I^3
uncoordinated movement							O_I^3

TABLE KEY:

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11 = unspecified DDD;
12 = DDT/DDE/DDD mixture

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: gallinaceous birds *(continued)*

Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Reproductive							
egg hatchability	O_I^4	NO_A^3 NO_I^{12} NO_C^4				NO_I^{12}	NO_I^3
egg production	NO_I^4	$O_I^{1,3,5}$ $NO_I^{1,5,12}$ NO_C^4		NO_I^1		NO_I^1 NO_A^3	NO_I^3
egg size/weight	O_I^4					NO_I^1	
egg fertility	O_I^4	NO_C^4					NO_I^3
timing of oviposition		$O_I^{1,3,5}$					
ovary weight		O_I^1 NO_A^2					
oviduct weight		NO_A^2 O_A^2				O_A^2	
testis weight		NO_I^2 O_I^1				O_I^4	
testicular atrophy						O_I^4	
semen volume						O_I^4	
percentage viable sperm						O_I^4	
sperm motility						O_I^4	
sperm concentration						O_I^4	
semen cholesterol						O_I^4	
testicular microscopic lesions						O_I^4	
gross gonad lesions							NO_I^3
decreased plasma estrogen						O_A^3	
uterine glycogen		O_A^2 NO_A^1				O_A^2 NO_A^1	

TABLE KEY:

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Subscript (Exposure Duration):
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Superscript (Chemical Identity):
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12 = DDT/DDE/DDD mixture

Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Birds: gallinaceous birds *(continued)*						
Adverse Biological Effect	Bobwhite quail	Japanese quail	California quail	Pharol D-1 quail	Crowned Guinea fowl	Domestic fowl	Ring-necked pheasant
Eggshell Thinning							
eggshell calcium		O_I^1 NO_I^5				NO_I^1	
eggshell thickness	O_I^4	$NO_I^{1,5}$		NO_I^1		NO_I^1 NO_A^3	
eggshell weight		$NO_I^{1,5}$		NO_I^1		NO_I^1	
egg breakage		O_I^3 $NO_I^{1,5,12}$				NO_I^{12}	
egg membrane thickness	O_I^4						
Developmental							
chick survival		O_A^3 NO_C^4					$O_I^{3,4}$
testis development						O_I^4	
secondary sex characteristics						O_I^4	
chick weight	NO_I^4	NO_C^4					
Genotoxic							
Cancer							

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

	Birds: passerines and non-passerine ground birds										
Adverse biological effect	House sparrow	Cowbird	Red-winged blackbird	Blue jay	Cardinal	Ringed turtle dove	Rock dove	Pigeons (homing and white king)	Bengalese finch	Redstart	White-throated sparrow
Mortality											
immature	O_A^3			O_A^3	O_A^3						
mature	O_A^4	O_A^1	$O_{A,I}^4$				O_A^4		O_I^1		
Systemic Effects											
Respiratory											
Cardiovascular											
pulse rate								O_I^1	O_I^1		
ventricular beat amplitude								O_I^1	NO_I^1		
heart weight								$O_I^{1,5}$	O_I^1		
heart muscle tone								O_I^5			
Gastrointestinal											
Hematological											
hematocrit						O_I^7					
Musculoskeletal											
soft skull								O_I^5			
calcium uptake in bone						O_I^1					
Hepatic											
liver weight						O_I^7		$O_I^{1,5}$		NO_A^1	
plasma aspartate aminotransferase						O_I^7					
vitamin A storage								O_I^1			
hypertrophy								O_I^1			
microsomal hormone metabolism						$O_I^{1,3}$		O_A^1			
Renal											
kidney weight										NO_A^1	

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: passerines and non-passerine ground birds *(continued)*

Adverse biological effect	House sparrow	Cowbird	Red-winged blackbird	Blue jay	Cardinal	Ringed turtle dove	Rock dove	Pigeons (homing and white king)	Bengalese finch	Redstart	White-throated sparrow
Endocrine											
adrenal weights								$O_I^{1,5}$			
thyroid weight								O_I^{1}			
hyperplasia of thyroid follicular epithelium								O_I^{1}			
decreased follicular colloid								O_I^{1}			
Dermal/ocular											
Body weight											
loss or reduced gain	O_A^{3}					NO_I^{7}		O_I^{5}		NO_A^{1}	O_I^{3} NO_I^{5}
decreased body fat											O_I^{3} NO_I^{5}
Metabolic rate											
body temperature								O_I^{1}			
oxygen consumption rate								O_I^{1}			
blood Na^+						O_I^{7}					
blood K^+						NO_I^{7}					
blood Ca^{+2}						NO_I^{7}					
Other											
activity of various dehydrogenases in various tissues										NO_A^{1}	
Immunological/ Lymphoreticular											

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: passerines and non-passerine ground birds *(continued)*

Adverse biological effect	House sparrow	Cowbird	Red-winged blackbird	Blue jay	Cardinal	Ringed turtle dove	Rock dove	Pigeons (homing and white king)	Bengalese finch	Redstart	White-throated sparrow
Neurological/Behavioral											
tremors	$O_A^{3,4}$	O_A^{1}		O_A^{3}	O_A^{3}			$O_I^{1,5}$			
balance disturbance	O_A^{3}			O_A^{3}	O_A^{3}						
affected courtship behavior						O_I^{5}					
						NO_I^{5}					
brain dopamine						O_I^{7}					
brain norepinephrine						O_I^{7}					
brain weight										NO_A^{1}	
migratory behavior											$O_I^{3,7}$
nesting behavior						O_I^{5}					
activity pattern										NO_A^{1}	
Reproductive											
egg hatchability						NO_I^{5}					
egg production						O_I^{5}					
						NO_I^{5}					
testis weight						NO_I^{5}			NO_I^{1}		
clutch size									NO_I^{1}		
ovary weight						O_I^{5}					
oviduct weight						O_I^{5}					
timing of oviposition						$O_I^{1,5}$			O_I^{1}		
egg size/weight						O_I^{1}			O_I^{1}		
serum LH levels						O_I^{5}					
blood estradiol levels						O_I^{1}					
Eggshell Thinning											
eggshell weight						O_A^{5}					
eggshell thickness						$O_I^{5,7}$					
^{45}Ca deposition in egg						O_I^{1}					
oviduct carbonic anhydrase activity						O_A^{5}					

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Table D-1. DDT/DDE/DDD Hazard Identification in Wildlife: Health Effects Observed in Experimental Toxicological Studies in Wildlife *(continued)*

Birds: passerines and non-passerine ground birds *(continued)*

Adverse biological effect	House sparrow	Cowbird	Red-winged blackbird	Blue jay	Cardinal	Ringed turtle dove	Rock dove	Pigeons (homing and white king)	Bengalese finch	Redstart	White-throated sparrow
Developmental											
chick early survival						O_I^5					
Genotoxic											
Cancer											

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APPENDIX E

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