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EFFECTS OF THE PESTICIDES CARBOFURAN, CHLORPYRIFOS, DIMETHOATE, LINDANE, TRIALLATE, TRIFLURALIN, 2,4-D, AND PENTACHLOROPHENOL ON THE METABOLIC ENDOCRINE AND REPRODUCTIVE ENDOCRINE SYSTEM IN EWES

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EFFECTS OF THE PESTICIDES CARBOFURAN, CHLORPYRIFOS, DIMETHOATE, LINDANE, TRIALLATE, TRIFLURALIN, 2,4-D, AND PENTACHLOROPHENOL ON THE METABOLIC ENDOCRINE AND REPRODUCTIVE ENDOCRINE SYSTEM IN EWES

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Many pesticides are used in the agricultural environment, and some may have the potential to disrupt reproductive or endocrine function. Ewes, in separate groups of 6, received orally into their rumen either empty gelatin capsules or capsules containing chlorpyrifos (12.5 mg/kg), trifluralin (17.5 mg/kg), lindane (2.5 mg/kg), or pentachlorophenol (2 mg/kg) 2 times per week for 43 d. Dimethoate (0.2 mg/kg), carbofuran (0.30 mg/kg), 2,4-dichlorophenoxyacetic acid (10 mg/kg), or triallate (5 mg/kg) was given 3 times per week. After 36 d of treatment, blood samples were taken every 12 min for 6 h for hormone analysis. Ewes were euthanized at the end of the study for necropsy and histopathology. No overt signs of toxicity were seen, and body weight was not affected by treatment. Carbofuran caused a significant increase in serum concentrations of thyroxine compared to control ewes, but all other pesticides, except trifluralin, resulted in a marked decrease in thyroxine concentrations. Serum concentrations of cortisol were significantly increased by trifluralin and chlorpyrifos. Concentrations of insulin in serum were markedly increased in ewes given dimethoate, lindane, trifluralin, triallate, and pentachlorophenol, and concentrations of estradiol were also significantly increased in ewes given lindane and trifluralin. Mean serum concentrations of LH were markedly decreased by trifluralin, and basal LH concentrations were significantly decreased by lindane, dimethoate, and trifluralin but increased by triallate. Both pentachlorophenol and triallate caused a significant increase in severity of oviductal intraepithelial cysts in ewes. Data suggest that several currently used pesticides could influence serum concentrations of reproductive and metabolic hormones, particularly thyroxine, the major secretory product of the thyroid and a principal regulator of metabolism.

Many pesticides are used in agriculture, and some may disrupt reproductive or endocrine function in farm animals, wild animals, and humans (Hayes & Laws, 1991; Colborn et al., 1993). However, for many pesticides, little information is available on the potential for endocrine disruption (Hayes & Laws, 1991; Colborn et al., 1993). In the present study, the

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ability of eight pesticides to affect the reproductive or endocrine system in ewes was examined. Sheep were used as a model species for farm animals and wild ungulates. The pesticides were chosen because they are present in agricultural areas or heavily used in agriculture (Spencer, 1982; Hayes & Laws, 1991; Ervine, personal communication, 1993) and represent a range of structures, persistencies, and toxicities. Pentachlorophenol is an organochlorine fungicide present in treated wood (Hayes & Laws, 1991). Triallate, 2,4-dichlorophenoxyacetic acid (2,4-D), and trifluralin are halogenated hydrocarbons used as herbicides (Hayes & Laws, 1991). The insecticides studied were lindane, an organochlorine; carbofuran, a carbamate; and dimethoate and chlorpyrifos, organophosphates (Hayes & Laws, 1991).

From a metabolic endocrine standpoint, there is information on the effects of pesticides on serum concentrations of thyroid hormones. It was suggested that many chlorinated hydrocarbons may displace T_4 from human serum thyroid binding proteins (Van den Berg et al., 1991). Pentachlorophenol has been shown to displace thyroxine (T_4) from human serum thyroid binding proteins (Van den Berg, 1990; Van den Berg et al., 1991) and also to decrease serum concentrations of the thyroid hormones (Hughes et al., 1985; Jekat et al., 1994) in calves and rats and thyroid-stimulating hormone concentrations in rats (Jekat et al., 1994). Serum concentrations of T_4 were reduced in rats by 2,4-D (Gorzinski et al., 1987), and this probably reflected effects on serum binding proteins and direct effects on the thyroid (Nicolau, 1983; Van den Berg et al., 1991). Carbofuran has been reported to increase serum concentrations of adrenal glucocorticoids in mice and rats, possibly by inhibiting metabolism by the liver (Cranmer et al., 1978). However, treatment with lindane resulted in increased serum concentrations of cortisol in rabbits (Anand et al., 1990) but reduced concentrations in mice (Lahiri & Sircar, 1991). In one study, dimethoate caused a decrease in adrenal and pituitary weights in rats (Shaker et al., 1988), and lindane has been shown to induce hypoglycemia in cats, suggesting a hyperinsulinemic state (Agrawal et al., 1987).

Apart from reports of fetotoxicity and reduced litter size from treatment of pregnant rats with pesticides, there are few reports of effects of pesticides on reproductive endocrine function in mature adults (Hayes & Laws, 1991; Colborn et al., 1993). In two studies in rats with chlorpyrifos, no effects were seen on reproduction (Quast et al., 1993). However, treatment with lindane resulted in disrupted estrous cycles in rats (Lahiri et al., 1985; Gray et al., 1988) and caused increased embryo loss (Sircar & Lahiri, 1989). Ovarian steroidogenesis appeared to be inhibited in mice by lindane (Sircar & Lahiri, 1990), and the embryo loss seen in rats was reversed by estradiol (Sircar & Lahiri, 1989). Lindane appears to be an antiestrogen (Chadwick et al., 1988; Cooper et al., 1989), but whether this is a direct effect on the estrogen receptor is unclear (Tezak et al., 1992; Laws et al., 1994).

Therefore, of the pesticides selected for study, based on agricultural use, only pentachlorophenol and lindane have been reasonably implicated as having effects on the metabolic and reproductive endocrine systems; however, most of this work was done in laboratory rodents. The specific goals of the present study were to look at the effects of pentachlorophenol, triallate, 2,4-dichlorophenoxyacetic acid, trifluralin, lindane, carbofuran, dimethoate, and chlorpyrifos on the pituitary, ovaries, adrenals, thyroid, pancreas, and serum concentrations of the hormonal products of these glands in mature ewes.

MATERIALS AND METHODS

Treatments and Sampling

Eighty 1- to 4-yr-old polypay ewes were kept in paddocks and fed a maintenance diet of alfalfa pellets with hay, minerals, and water available ad libitum. Estrus was detected by four color marker harnessed rams (Net Tex Agric. Ltd., Gravesend, UK). Sixty-six ewes that had had 2 consecutive periods of estrus were divided into 11 groups; groups were age and weight matched. Each experimental group contained 2 ewes 1 yr of age, 2 ewes 1–2 yr of age, and 2 ewes 3–4 yr of age or older, based on eruption of teeth. During the mid breeding season (October–November), ewes were brought inside and each group of ewes was placed in a 4 × 4 m pen under fluorescent lighting, with photoperiod adjusted weekly to ambient day length (sunrise to sunset), plus one-half hour for twilight. Ewes were treated with gelatin capsules containing either dimethoate (Cygon 240EC, Ciba Geigy Canada Ltd., Mississauga, ON), carbofuran (Furalan, Chem Agro, Etobicoke, ON), chlorpyrifos (Lorsban 4E, Dow-Elanco Chemical Co., Sarnia, ON), 2,4-D (Dow-Elanco), trifluralin (Treflan, Dow-Elanco), triallate (Avadex, BW, Monsanto, St. Louis, MO), lindane (Lindane, 10% EC, Sanex Agro Inc., Dundas, ON), or pentachlorophenol (PCP, 99.9% pure, Sigma Chemical Co., Mississauga, ON) for 43 d. Apart from PCP, all of the pesticides were used as commercial preparations.

Doses and dose frequencies are shown in Table 1. Lowest observed adverse effect levels (LOAEL) and no observed adverse effect levels (NOAEL) for ruminants were obtained from the literature (Spencer, 1982; Ecobichon, 1991; Hayes & Laws, 1991). Little information was available and what was found was quite variable; therefore, we verified these results in ewes in preliminary studies. Using the range of doses given in the literature for LOAEL and NOAEL we treated (as described earlier) separate groups of 2 ewes once, with each of the pesticides at 3 doses, each separated by a factor of 10. In every case, the NOAEL was further confirmed by a fourth, intermediate dose of pesticide. Heart rate, respiration rate, and temperature were measured 30 min prior to treatment and every 30 min after treatment for 3 h and then hourly for a further 3 h. Ewes

TABLE 1. Doses, dose schedule, and experimental section for ewes given 1 of 8 pesticides orally for 43 d

Pesticide	Dose (mg/kg)	Doses per week[a]	Experimental section[b]
Dimethoate	0.2	3	1
Carbofuran	0.3	3	3
Chlorpyrifos	12.5	2	2
2,4-D	10	3	2
Trifluralin	17.5	2	1
Triallate	5	3	2
Lindane	2.5	2	1
Pentachlorophenol	2	2	3

[a]Monday and Friday or Monday, Wednesday, and Friday.
[b]Sections 1, 2, and 3 were started 11 d apart.

were under constant observation for the initial 3 h, checked hourly for a further 24 h, and then daily for several days. Any toxic effects, such as changes in salivation, respiration, temperature or heart rate, motor function, activity, reactivity, feed intake, defecation, or urination, were recorded. Doses in the present longer term study were designed to be at least 10-fold lower than the acute no-observed-adverse-effect levels, to avoid any acute toxicity and yet keep doses high enough to see effects of chronic exposure to pesticides on the endocrine system. Pesticides with some degree of persistency or that could bioaccumulate were given twice weekly; other pesticides were given three times weekly (Table 1; Hayes & Laws, 1991). Gelatin capsules were given orally by balling gun directly

TABLE 2. Severity scores for oviducal intraepithelial cysts in ewes treated with 1 of 8 pesticides for 43 d

	Oviduct intraepithelial cysts	
Pesticide	Right	Left
Control	1.24 ± 0.31	1.10 ± 0.29
Lindane	0.79 ± 0.20	1.42 ± 0.19
Dimethoate	1.75 ± 0.23	1.58 ± 0.28
Trifluralin	1.75 ± 0.23	1.75 ± 0.33
2,4-D	1.25 ± 0.20	1.42 ± 0.30
Triallate	1.96 ± 0.46	2.58 ± 0.15[a]
Chlorpyrifos	2.08 ± 0.19	1.63 ± 0.40
PCP	2.42 ± 0.19[a]	2.75 ± 0.33[a]
Carbofuran	1.79 ± 0.43	1.75 ± 0.33

Note. See text for doses and dose schedules. Cysts were scored as minimal (0–1), mild (1–2), moderate (2–3), or marked (3–4), and within each category as focal (0.25), diffuse (0.50), or multifocal (0.75).
[a]Significantly different from control ($p < .05$).

into the rumen; control ewes received empty capsules. To facilitate handling and experimental treatments, the study was broken down into 3 sections, with sections started 11 d apart. The treatments in each section are shown in Table 1. There was a group of control ewes in each section.

All ewes were weighed once weekly and blood samples were taken by jugular venipuncture twice weekly (10 ml, Tuesday and Friday). After 36 d of treatment, ewes were bled every 12 min (4 ml) for 6 h from a jugular catheter fitted 1 d previously (vinyl catheter; ID 1 mm, OD 1.5 mm; Dural Plastics and Engineering, Dural, NSW, Australia). This intensive bleeding was necessary to accurately assess serum concentrations of some hormones that are secreted in a pulsatile manner. This blood sampling was done when ewes were at d 8–10 of an estrous cycle to ensure that serum concentrations of reproductive hormones did not vary due to the stage of estrous cycle. To ensure all ewes were at this stage of the cycle, estrus was synchronized by a 12-d treatment with intravaginal sponges containing 60 mg medroxy progesterone acetate (Veramix: Tuco Products Company, Orangeville, Ontario, Canada). Sponges were inserted on d 14 of treatment and removed on d 26. As ewes were inside when estrus was synchronized, rams were not used to detect estrus, but synchronizing of the estrous cycles was confirmed by measuring progesterone in the blood samples collected twice weekly. All blood samples were allowed to clot at room temperature overnight; blood clots were removed and serum centrifuged at 3000 × g for 15 min, and serum was decanted and stored at –20°C until analyzed.

Histopathology

After 43 d of treatment, ewes were euthanized with Euthanyl Forte (MTC Pharmaceuticals, Cambridge, Ontario, Canada). At necropsy, samples of the thyroid, adrenals, pancreas, pituitary, thymus, ovaries, oviducts, uterus, brain, bone marrow, lymph nodes (mediastinal and mesenteric), lung, liver, heart, kidneys, and spleen were fixed in normal buffered formalin. Tissues were stained with hematoxylin and eosin for histopathological examination. Each tissue was examined for any potential treatment-related lesions. Abnormalities were scored as minimal (0–1), mild (1–2), moderate (2–3), or marked (3–4). Within each category, severity was also scored as focal (0.25), diffuse (0.50), or multifocal (0.75).

Hormone Analysis

All of the blood samples collected every 12 min for 6 h were analyzed for concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by previously validated radioimmunoassays (Joseph et al., 1992). Concentrations of LH and FSH are expressed in terms of NIDDK-oLH-24 and NIDDK-oFSH-RP1, respectively. Assay sensitivity, as assessed by the lowest standard different from zero (*t*-test), was 0.25 ng/ml and 0.06 ng/ml for FSH and LH, respectively. Intra- and interassay

coefficients of variation (CVs) for an ovine reference serum with mean FSH concentrations of 0.73 ng/ml were 9 and 4%, respectively. Intra- and interassay coefficients of variation for an ovine reference serum with mean LH concentrations of 2.77 ng/ml were 6 and 7%, respectively. Samples were pooled for each hour of the intensive bleed and analyzed for concentrations of progesterone, estradiol, thyroxine (T_4), cortisol, and insulin by validated immunoassays. Progesterone and estradiol were measured by radioimmunoassays (Joseph et al., 1992, 1994) with assay sensitivities of 0.16 nmol/L and 3.67 pmol/L, respectively. Intra- and interassay coefficients of variation were 9 and 14% (mean 6.4 nmol/L) or 9 and 13% (mean 32.66 pmol/L) for progesterone and estradiol, respectively. Thyroxine (T_4) and cortisol (Kingsbury & Rawlings, 1993) were measured using a fluorescence polarization immunoassay (TD_x systems, Abbott Laboratories, Irving, TX) with assay sensitivities of 5 nmol/L and 12.4 nmol/L, respectively. Intra- and interassay coefficients of variation were 6 and 9% (mean 54.5 nmol/L) or 16 and 13% (mean 40.9 nmol/L) for thyroxine and cortisol, respectively. When 17 nmol/L of T_4 was added to ovine serum, a value of 15 nmol/L was obtained after subtraction of the endogenous concentration of T_4 (85 nmol/L). Insulin was measured by radioimmunoassay (Mears et al., 1988), and insulin concentrations are expressed in terms of ovine reference insulin I-9254 (Sigma Chemical Co., St. Louis, MO). Assay sensitivity was 0.06 ng/ml and the intra- and interassay coefficients of variation were 12 and 15% (mean 0.23 ng/ml) or 11 and 13% (mean 0.90 ng/ml), respectively.

Statistical Analysis

For blood samples collected every 12 min for 6 h, the pulsar program (Merriam & Wachter, 1982) was used to identify pulses of LH and FSH secretion. LH data are presented as pulse frequency and amplitude and mean and basal concentrations in serum. Basal concentrations are those after the removal of pulses. Serum concentrations of FSH were judged not to be pulsatile and therefore only mean serum concentrations were analyzed. For each analysis, control values from each experimental section were combined if the control values did not differ between sections. When control values did vary between sections, comparisons of control and treatment groups were made within sections. All data are presented as mean and standard error of the mean. Hormone data were compared between treatment and control groups by analysis of variance ($p < .05$) and Student–Newman–Keuls procedure ($p < .05$; Sokal & Rohlf, 1969). The severity of histopathological findings was examined by analysis of variance and Dunnett's procedure ($p < .01$; Sokal & Rohlf, 1969).

RESULTS

No overt signs of toxicity were seen during the study. Body weight was not markedly affected by treatment; mean body weight for all ani-

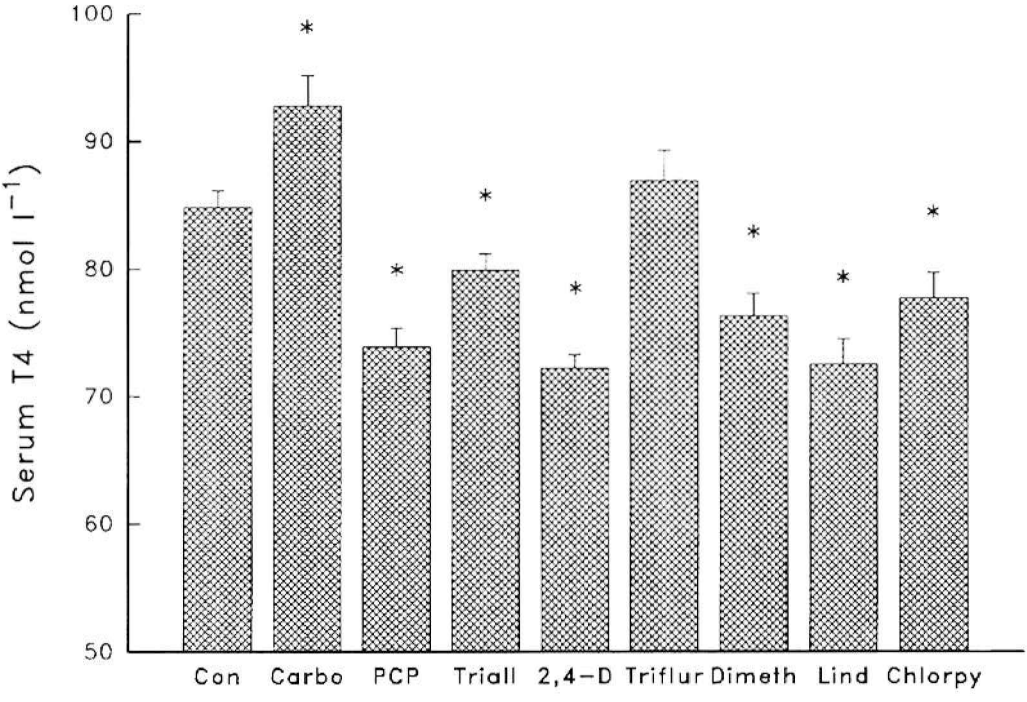

FIGURE 1. Mean serum concentrations of thyroxine (T_4) in ewes based on blood samples collected every hour for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). Asterisks show significant differences from the control ewes ($p < .05$).

mals was 51.3 ± 0.8 kg at the beginning of treatment and 54.7 ± 0.8 kg at the end of treatment. On histopathological examination of tissues the only treatment-specific effect was an increased severity of oviducal intraepithelial cysts in ewes treated with PCP (both oviducts) and triallate (left oviduct only) compared to control ewes.

In control ewes, serum concentrations of estradiol increased significantly from experimental section 1 to 3, concentrations of insulin increased from section 1 to 2, but basal and mean serum concentrations of LH were significantly lower in control ewes of sections 2 and 3 compared to section 1 (see Figures 3–6).

Carbofuran treatment resulted in a significant increase in serum concentrations of T_4 compared to control ewes in samples taken at the intensive bleed after 36 d of treatment, but all other pesticides, except trifluralin, resulted in a marked decrease in T_4 concentrations (Figure 1). Serum concentrations of cortisol were significantly increased by trifluralin and chlorpyrifos (Figure 2). Concentrations of insulin in serum were markedly increased in ewes given dimethoate, lindane, trifluralin, triallate, and PCP (Figure 3), and concentrations of estradiol were also increased in ewes treated with lindane and trifluralin (Figure 4). LH pulse

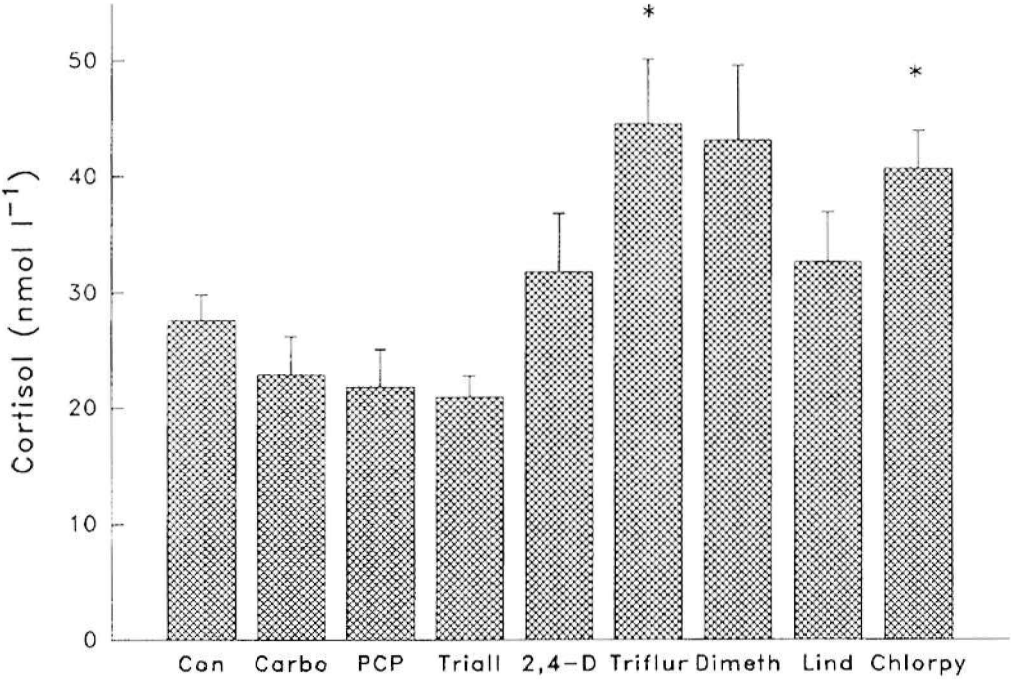

FIGURE 2. Mean serum concentrations of cortisol in ewes based on blood samples taken every hour for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). Asterisks show significant differences from the control ewes ($p < .05$).

frequency (mean for all ewes, 0.37 ± 0.04 pulses/h) and amplitude (0.61 ± 0.02 ng/ml) and mean serum concentrations of FSH (2.95 ± 0.26 ng/ml) and progesterone (1.59 ± 0.09 ng/ml) were not markedly affected by treatment. Mean serum concentrations of LH were significantly decreased in ewes given trifluralin (Figure 5), and basal LH concentrations were markedly decreased by lindane, dimethoate, and trifluralin but increased by treatment with triallate (Figure 6).

DISCUSSION

In a study of thyroid hormone binding in human serum (Van den Berg et al., 1991), 60% of 65 industrial chemicals were shown to have some ability to displace T_4 and reduce serum concentrations of T_4; chlorinated hydrocarbons such as pentachlorophenol and 2,4-D were particularly effective. Although some of the very avid thyroid-hormone-binding proteins found in humans are not present in other species (Capen & Martin, 1989), competition for binding proteins may provide a partial explanation for the reduced serum concentrations of T_4 caused by pentachlorophenol, triallate, 2,4-D, dimethoate, lindane, and chlorpyrifos in the present study. In previous studies, 2,4-D and PCP caused a reduction in serum T_4

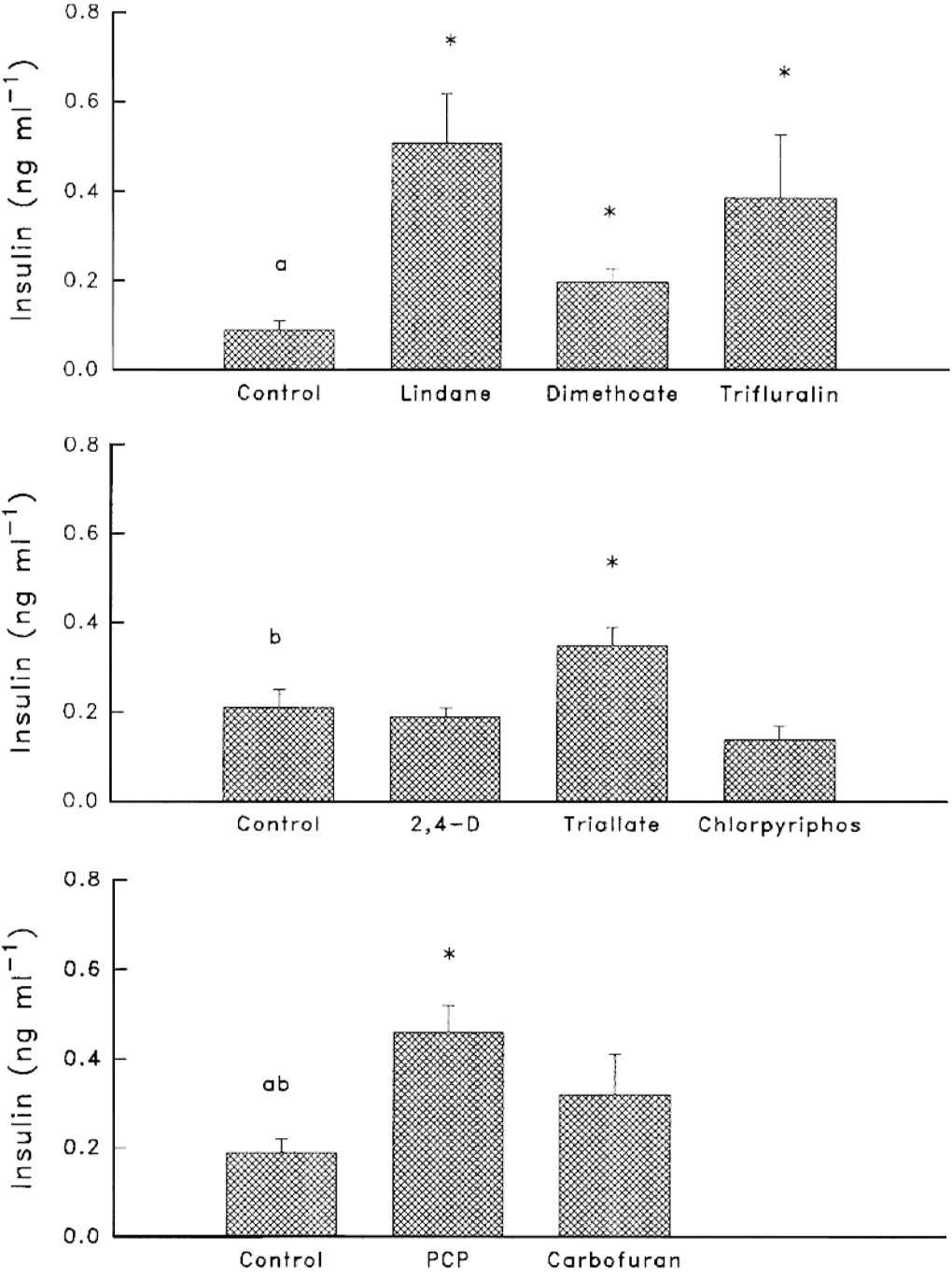

FIGURE 3. Mean serum concentrations of insulin in ewes based on blood samples taken every hour for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). The study was run in three sections. Asterisks indicate significant differences between pesticide treatment groups and controls within each section ($p < .05$) and letters indicate significant differences between the control groups.

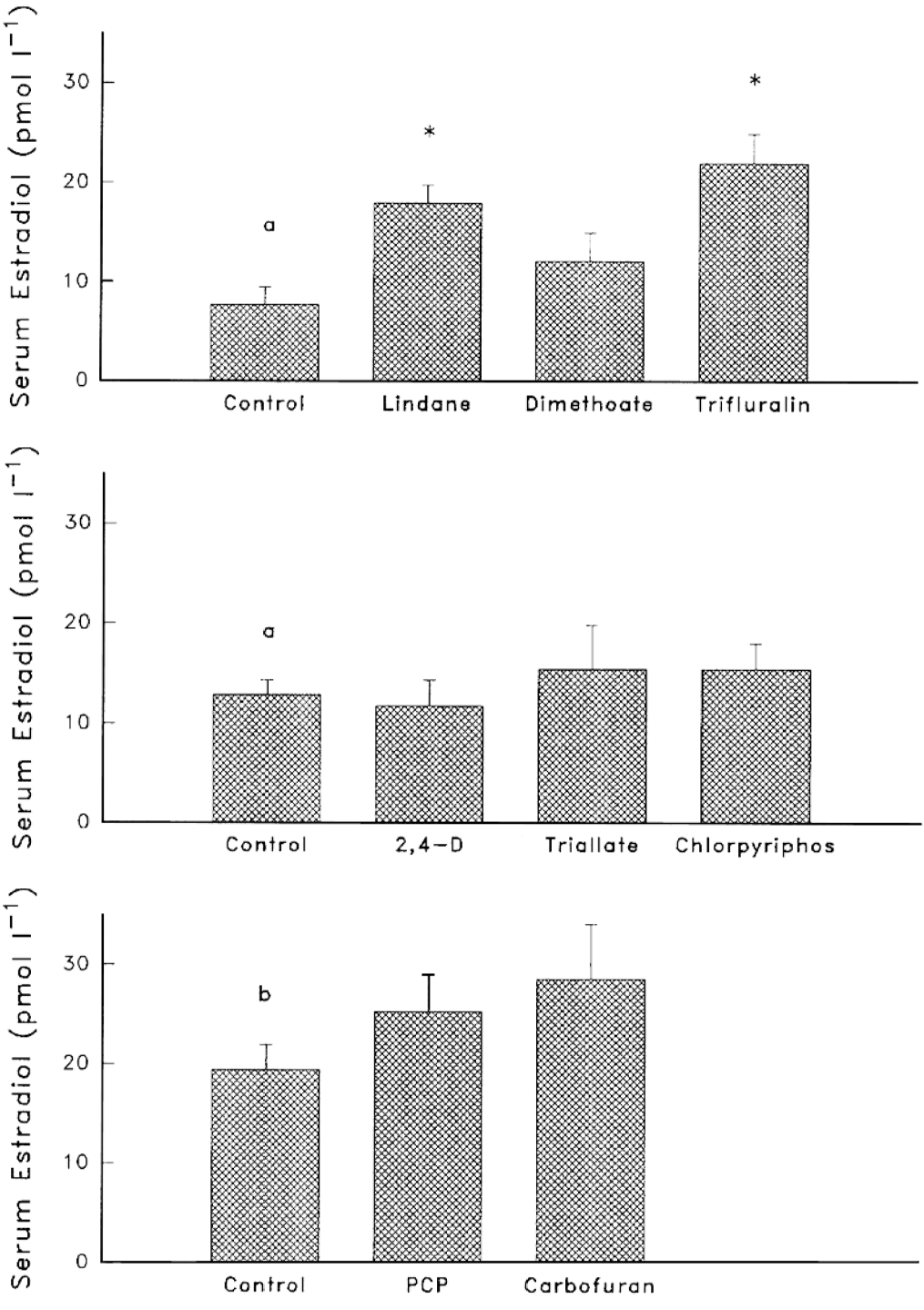

FIGURE 4. Mean serum concentrations of estradiol in ewes based on blood samples taken every hour for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). The study was run in three sections. Asterisks indicate significant differences between pesticide treatment groups and controls within each section ($p < .05$) and letters indicate significant differences between the control groups.

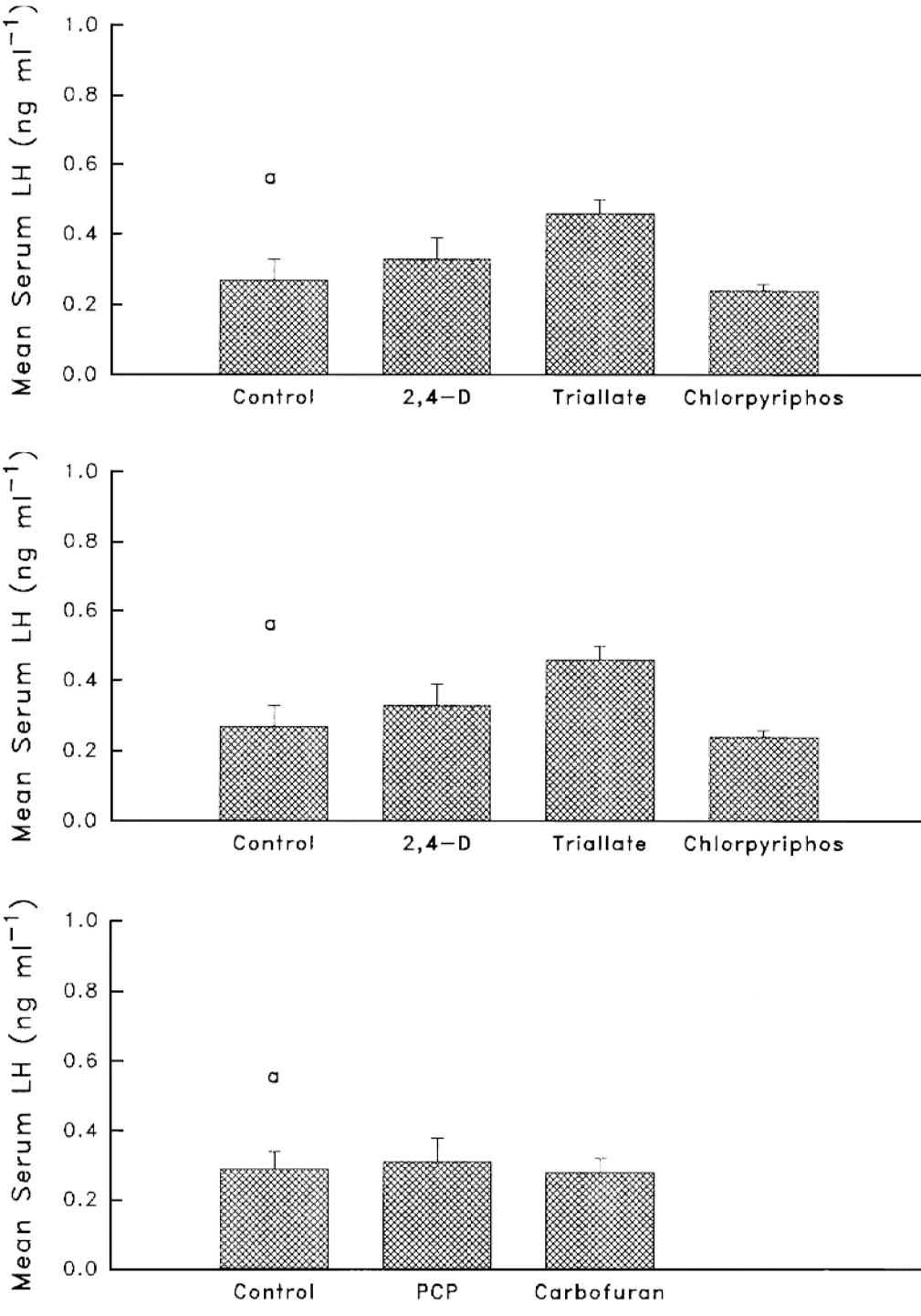

FIGURE 5. Mean serum concentrations of LH in ewes based on blood samples taken every 12 min for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). The study was run in three sections. Asterisks indicate significant differences between pesticide treatment groups and controls within each section ($p < .05$) and letters indicate significant differences between the control groups.

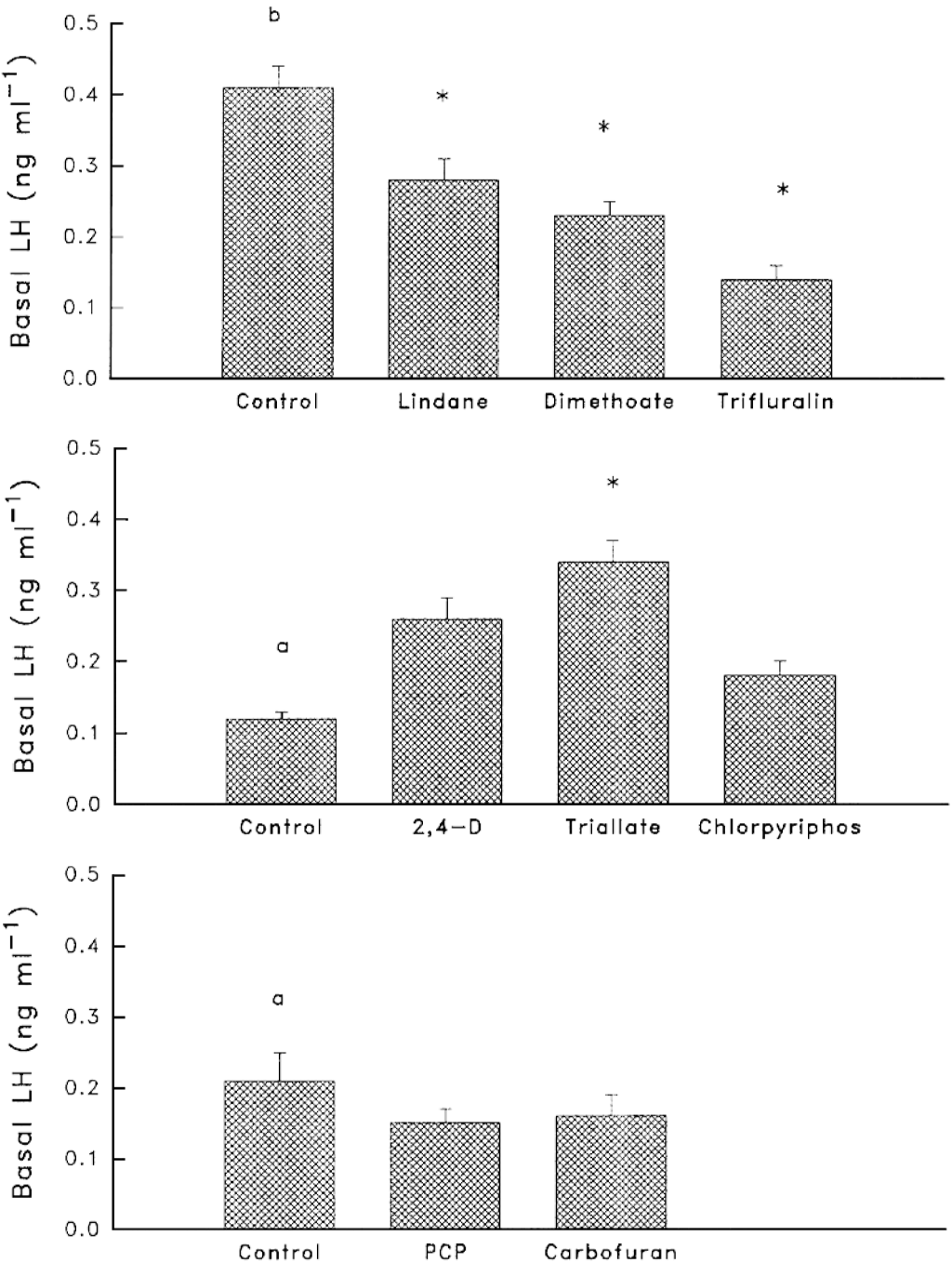

FIGURE 6. Mean basal serum concentrations of LH in ewes based on blood samples taken every 12 min for 6 h after 36 d of treatment with 1 of 8 different pesticides (see text for details of treatments). The study was run in three sections. Asterisks indicate significant differences between pesticide treatment groups and controls within each section ($p < .05$) and letters indicate significant differences between the control groups.

concentrations in rats (Gorzinski et al., 1987; Jekat et al., 1994) and PCP caused a reduction in calves (Hughes et al., 1985). In rats these results were at least partly due to direct effects at the thyroid (Nicolau, 1983; Jekat et al., 1994) and perhaps even the pituitary (Jekat et al., 1994). However, both pentachlorophenol and lindane have been shown to induce liver microsomal enzymes (Street & Chadwick, 1975; Schulte & Parzefall, 1980), which could result in enhanced metabolism of T_4. Inhibition of the ability of the liver to metabolize corticosteroids by carbofuran (Cranmer et al., 1978) may indicate a general inhibition of liver metabolic function, which could explain the increased serum concentrations of T_4 seen in ewes treated with carbofuran.

Serum concentrations of cortisol can be quite variable as the hypothalamic–pituitary–adrenal axis is very much influenced by stress (Hsu & Crump, 1989). Carbofuran has been shown to increase serum concentrations of adrenal glucocorticoids (Cranmer et al., 1978) in mice and rats, but an effect was not seen in ewes. Lindane was reported to increase serum concentrations of cortisol in rabbits but to reduce concentrations of cortisol in mice (Anand et al., 1990; Lahiri & Sircar, 1991). Increased serum concentrations of cortisol in response to treatment with trifluralin or chlorpyrifos, as seen in the present work with ewes, have not been reported previously. The mechanism of the effects of trifluralin and chlorpyrifos is unclear, but a simple stress effect cannot be ruled out.

Interestingly, ewes in the five treatment groups that had increased serum concentrations of insulin (lindane, dimethoate, trifluralin, triallate, and pentachlorophenol) also had numerically greater concentrations of estradiol compared to control ewes, although in only two of these groups (lindane and triluralin) were serum estradiol concentrations significantly higher than in control ewes. It has been shown that insulin and insulin-like growth factors enhance ovarian follicle granulosa cell function and estradiol production (Roche, 1996). The pesticide-induced changes in serum concentrations of estradiol seen in the present study did not appear to be caused by changes in LH or FSH secretion. We are not aware of previous reports of direct effects of pesticides on pancreatic function; however, the present results suggest that lindane and trifluralin may increase estradiol secretion by enhancing insulin production.

Lindane, pentachlorophenol, and 2,4-D have been reported to reduce fertility in female rats (Exon & Koller, 1982; Mohammad & St. Omer, 1986; Sircar & Lahiri, 1989). Embryo loss in lindane-treated rats was reversed by treatment with estradiol (Sircar & Lahiri, 1989), and lindane inhibited cholesterol side-chain cleavage in the mouse ovary (Sircar & Lahiri, 1990). It was suggested that lindane has both estrogenic (Lahiri et al., 1985) and antiestrogenic effects (Chadwick et a., 1988; Cooper et al., 1989). Although lindane does not appear to alter estradiol receptor numbers or affinity (Laws et al., 1994), it can compete with estradiol for binding to the estradiol receptor (Tezak et al, 1992). No evidence of direct

estrogenic or antiestrogenic effects of lindane in ewes was seen. In contrast to the inhibition of steroidogenesis seen in mice (Sircar & Lahiri, 1989), the results of the present study suggested an indirect stimulation of estradiol secretion in ewes by lindane, involving increased secretion of insulin. In previous studies, lindane treatment resulted in a hypoglycemic condition in cats and increased body weight in rats (Chadwick et al., 1988; Gray et al., 1988), suggesting an increased secretion of insulin.

Our findings in sheep supported previous observations of decreased LH secretion in rats (Lahiri et al., 1985) and humans (Sircar & Lahiri, 1989) treated with lindane. However, there were few previous observations to compare with the decreased LH secretion seen in ewes treated with dimethoate or trifluralin and the increased basal serum concentrations of LH noted in ewes given triallate. In one previous study pituitary weights were reduced in rats treated with dimethoate (Shaker et al., 1988); no pituitary pathology was noted in the present study.

The decrease in mean and basal serum concentrations of LH in control ewes, from the first to the third section of the experiment, probably reflected a seasonal change in LH secretion (Currie et al., 1993). From early to mid breeding season, the period in which the study was done, frequency of secretion of pulses of LH increases (Currie et al., 1993), which could give a decrease in basal LH secretion. In the present study, in control ewes, LH pulse frequency appeared to increase from the first (0.25 ± 0.04) to the second section (0.36 ± 0.04) of the study, but this numerical difference was not significant. As LH pulse frequency is low (0.31 ± 0.02 pulses/h) in ewes at the stage of the estrous cycle we studied, mean serum LH would largely be influenced by the basal LH concentration. Therefore, the decrease in mean serum LH concentration in control ewes over the course of our studies probably reflected similar changes in basal LH concentration. It is known that increased LH pulse frequency stimulates ovarian follicular estradiol production (Goodman, 1994), and this may explain the increased serum concentration of estradiol from the first to the third section of our study.

Few histopathological abnormalities were seen. Both triallate and pentachlorophenol caused an increase in oviductal intraepithelial cysts, but these two pesticides did not produce endocrine changes that were unique to these two pesticides and that could be specifically related to the incidence of intraepithelial cysts. This lesion is considered to be unusual, especially to the degree observed here (McEntee, 1990). None of the endocrine changes seen were accompanied by histopathological changes in endocrine or target tissues; however, our treatment period was only 6 wk.

The pesticides studied are currently used in agriculture and industry and vary in persistency (Hayes & Laws, 1991). It is unlikely that wild or domestic mammals would be chronically exposed to the doses of pesticides used in this study, but there is a real concern that in agricultural

areas, particularly in the growing season, wild animals especially could be exposed to dozens or even hundreds of industrial chemicals (Colborn et al., 1993). The potential for many chemicals to disrupt endocrine systems over the long term—for instance, the thyroid system (Van den Berg et al., 1991)—could reduce animal growth, reproduction, productivity and survivability. Even small perturbations in an endocrine system like the thyroid could reduce an animal's ability to successfully respond to stress and adverse environmental conditions.

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