Effects of an organophosphate pesticide, quinalphos, on the hypothalmo–pituitary–gonadal axis in adult male rats

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The effects of chronic sub-lethal doses (7–14 mg kg\(^{-1}\) a day for 15 days) of quinalphos were evaluated in adult male rats for changes in testicular morphology, circulatory concentrations of hormones (LH, FSH, prolactin and testosterone), activities of acetylcholinesterase (AChE) and angiotensin converting enzyme (ACE) as well as metabolism of biogenic amines (dopamine, noradrenaline and 5-hydroxytryptamine (5-HT)) in the hypothalamus and pituitary. Hormones were assayed by radioimmunoassay or chemiluminescent immunoassay (testosterone). The enzymes were estimated after spectrophotometry and the biogenic amines by HPLC–electrochemistry. Sub-lethal chronic administration of quinalphos resulted in: decreased testicular mass and AChE activity in central as well as peripheral organs; increased serum LH, FSH, prolactin and testosterone concentrations; decreased pituitary or increased testicular ACE activity; severe disruption of spermatogenesis with increasing doses of pesticide; and no significant effects on dopamine, noradrenaline or 5-HT concentrations in the hypothalamus or pituitary. Administration of oestradiol (50 µg per rat a day) during pesticide treatment resulted in: a significant decrease in the mass of the testis and accessory sex organs; decreases in serum LH, FSH, testosterone concentrations; an increase in prolactin concentration; and a decrease in dopamine or an increase in noradrenaline and 5-HT in the hypothalamus or pituitary. Oestradiol had a marked effect: in pesticide-treated animals, the pesticide effects were significantly reversed. This indicates that in pesticide toxicity, the hypothalamo–pituitary–gonadal axis is operational. Since many of the observed pesticide effects could be inhibited by oestradiol, it is suggested that the pesticide acts directly on the gonadotrophins. In conclusion, quinalphos decreases fertility in adult male rats by affecting the pituitary gonadotrophins.

Introduction

Studies on the biocidal effects of organophosphate pesticides are of immense importance in the field of toxicology. Organophosphates are chosen on the basis of their properties of low bioaccumulation and high rate of biodegradation (Rattner et al., 1982). Environmental pollution from organophosphate pesticides is an important issue that attracts widespread public concern. Owing to the extensive use of organophosphate pesticides in agriculture, there is a high risk of human exposure to these chemicals. The risk of acute exposure to these compounds is a constant threat and they are responsible for numerous cases of poisoning annually in non-target wildlife and for acute mammalian toxicity and neurotoxicity (Laham et al., 1986; Fautz and Milttenburger, 1994). In mammals, the primary site of action of organophosphate pesticides is the central and peripheral nervous system as they inhibit acetylcholinesterase (AChE) (Civen et al., 1977; Briggs and Simons, 1986), the enzyme that hydrolyses the neurotransmitter acetylcholine (ACh). In the presence of an inhibitor of AChE, synaptic acetylcholine may increase to abnormally high concentrations, which is postulated to precipitate a 'cholinergic crisis' that can be debilitating and possibly fatal (Ecobichon, 1991; Padilla et al., 1994). In surviving animals, several physiological and behavioural dysfunctions persist after exposure to high doses of organophosphates (Hall and Clark, 1982; Rattner et al., 1987). Sub-lethal doses of these pesticides lead to alterations in reproductive performance in birds and mammals (Somkuti et al., 1987; Ray et al., 1987, 1991; Chapin et al., 1990; Maitra and Sarkar, 1995, 1996). However, the mechanism of organophosphate-induced gonadal dysfunction remains to be elucidated.

There are several possible mechanisms for the anti-gonadal actions of organophosphates: they may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophin concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of neurotransmitters. In...
the present study, changes in the enzymatic and hormonal milieu after organophosphate treatment were investigated.

Materials and Methods

**Materials**

Quinalphos [\(\text{o-o-diethyl-o-(2-quinoliny1 phosphorothioate)}\)] was purchased from a local supplier as ‘Suquin’ 25% EC (Sudarshan Chemical Industries Ltd, Pune). Acetylthiocholine iodide, 5,5-dithio-bis-nitrobenzoic acid (DTNB), chloramine-T, heptane sulfonic acid, 5-hydroxtryptamine (5-HT), noradrenaline, dopamine, 3,4-dihydroxyphenyl acetic acid (DOPAC), homovanillic acid, 5-hydroxyindoleacetic acid (5-HIAA), angiotensin-converting enzyme (ACE) and hippuryl-L-histidyl-L-leucine were purchased from Sigma Chemical Co. (St Louis, MO). Testosterone chemiluminescent immunoassay kit (ACS 180 kit) was purchased from Coming Diagnostics Corp., East Walpole, MA. Radioimmunoassay reagents for prolactin, LH and FSH were obtained from the National Hormone and Pituitary Programme (NIDDK, Bethesda, MD). Labelled iodine (\(^{125}\text{I}\)) was procured from Bhabha Atomic Research Centre, Bombay. Oestradiol (1,3,5(10)-oestratrien 3,17-biol 3-benzoate) was purchased from Steraloid (Newport, RI). All other reagents were purchased locally and were of analytical grade.

**Animals and experimental design**

Adult male Sprague–Dawley rats weighing 200–250 g were obtained from the animal facility of the Institute. The animals were housed three per cage under controlled conditions of temperature (22 ± 1°C), humidity (60 ± 5%) and light (12 h light: 12 h dark cycle) with free access to food pellets and water. The experimental protocol met the national guidelines on the proper care and use of animals in laboratory research (Indian National Science Academy, New Delhi) and was approved by the animal ethics committee of the Institute. Quinalphos was diluted in olive oil. The pesticide doses of quinalphos were determined by observing the behaviour of the animals. The doses were selected on the basis of perceptible (7 mg kg\(^{-1}\)), mild (10 mg kg\(^{-1}\)) or moderate (14 mg kg\(^{-1}\)) behavioural manifestations. Animals were divided into six groups (\(n = 6\) per group). They were administered the vehicle (group I: 100 µl olive oil) or quinalphos (groups II–IV: 7, 10 or 14 mg kg\(^{-1}\) in 100 µl oil) by gavage once a day for 15 consecutive days.

Animals in groups V and VI were studied for effects of oestradiol (50 µg per rat in 100 µl olive oil) alone (group V) or in combination with quinalphos (group VI: 7 mg kg\(^{-1}\)) administered once a day for 15 days. The aim was to determine whether the pesticide affects the gonado–pituitary axis. By challenging with oestradiol, it was possible to confirm the functional status of the gonado–pituitary axis. At 1 day after the last day of treatment, all the animals were killed between 10:00 and 12:00 h to avoid any diurnal fluctuation in the concentrations of hormones and neurotransmitters. Body weight was recorded before and after the treatments.

Blood was collected by cardiac puncture under light ether anaesthesia. Care was taken not to increase the ether inhalation, to prevent any interference with the neurotransmitters. Serum was stored at –20°C for immunoassays of LH, FSH, prolactin and testosterone. Hypothalamus, pituitary, seminal vesicles, ventral prostate and paired testes were quickly dissected out and weighed. One of the testes was fixed in Bouin’s fixative for histological evaluation and the other testis was used for the enzyme assays.

**Histology**

The testis fixed in Bouin’s fixative was processed for paraffin wax sectioning (5 µm) and stained with periodic acid–Schiff (PAS) and haematoxylin.

**Enzyme assays**

Hypothalamus, pituitary and one part of the right testis were homogenized separately with a Potter-Elvehjem homogenizer. The other half of the right testis was homogenized in 100 mmol potassium phosphate buffer l\(^{-1}\) containing 500 mmol NaCl l\(^{-1}\) (pH 8.5) for estimation of ACE. The homogenates were centrifuged at 10 000 g at 4°C for 30 min and the supernatant was used for estimation of AChE and ACE.

AChE activity was determined according to the method of Ellman et al. (1961). Briefly, the rate of hydrolysis of acetylthiocholine in the presence of DTNB was recorded at 412 nm. The reaction mixture contained 0.1 mol sodium phosphate buffer l\(^{-1}\) (pH 8.0), DTNB (10 mmol l\(^{-1}\)), acetylthiocholine iodide (75 mmol l\(^{-1}\)) and tissue homogenate (400 µl). The rate of increase of absorbance was recorded in an LKB Ultraspec II spectrophotometer and the unit of enzyme activity was expressed as nmol per min per mg protein, taking the extinction coefficient of the yellow anion formed as \(E_{280} = 5.74 \times 10^{4}\).

ACE activity was determined by measuring the production of hippuric acid from hippuryl-L-histidyl-L-leucine according to the method of Cushman and Cheung (1971). Briefly, the assay mixture containing potassium phosphate buffer (100 mmol l\(^{-1}\)), NaCl (300 mmol l\(^{-1}\)), hippuryl-L-histidyl-L-leucine (5 mmol l\(^{-1}\)) and enzyme (0–10 mU) or tissue homogenate was incubated for 30 min at 37°C with shaking. The enzyme reaction was terminated by adding HCl (1 mol l\(^{-1}\)). The product formed by the enzymatic reaction, hippuric acid, was extracted from the acidified solution into 1.5 ml ethyl acetate and 1.0 ml distilled water and the amount of the product was measured at 228 nm. The enzyme activity was defined as nmol hippuric acid per min per mg protein.

**Radioimmunoassays of hormones**

Serum prolactin, LH and FSH were measured by using a double antibody radioimmunoassay (Chowdhury et al.,
Highly purified rat prolactin (rPRL-I-6), rat LH (rLH-I-4) and rat FSH (rFSH-I-8) were iodinated with 1 mCi $^{125}$I and freshly prepared chloramine T. For the prolactin assay, NIDDK rPRL-RP-3 was used as a standard and NIDDK rPRL antiserum S-9 was used for the assay. The limit of detection of the assay was 0.1 ng at 90% and the intra-assay coefficient of variation was 3.0%. NIDDK rLH-RP-3 was used for the LH assay. The limit of detection of rLH was 0.05 ng at 80%. For the FSH assay, NIDDK rFSH-RP-2 was used as a standard and NIDDK anti-rFSH-S-11 was used for the assay. The limit of detection was 0.04 ng at 98%. All the samples were assayed on the same day to avoid interassay variation. Intra-assay variation was 3.5%.

Serum testosterone was assayed using a competitive chemiluminescent immunoassay kit. The testosterone in the sample competes with acridium ester-labelled testosterone for binding to polyclonal rabbit and testosterone antibody in the solid phase. The polyclonal rabbit anti-testosterone antibody is bound to monoclonal mouse anti-rabbit antibody, which is coupled to paramagnetic particles. The assay uses a testosterone-releasing agent to release bound testosterone from the endogenous binding proteins. There is an inverse relationship between the amount of relative light units detected by the ACS:180 system. The ACS testosterone assay shows high specificity for testosterone. It shows 5.4% crossreactivity with 5-$\alpha$-dihydrotestosterone when 100 ng ml$^{-1}$ is present as contaminant. The minimum detectable range of the assay is 10 ng dl$^{-1}$. Maximum intra-assay variation was 11.3%.

HPLC assay of neurotransmitters and their metabolites

Pituitary and hypothalamic tissues were weighed and sonicated in 0.1 mol ice-cold perchloric acid $^{14}$ containing 0.05% EDTA. They were centrifuged at 10 000 $g$ for 10 min at 4°C and 10 $\mu l$ of the supernatant was injected directly into an HPLC system (Waters, Milford, MA) to determine noradrenaline, dopamine, 5-HT, DOPAC, homovanillic acid (HVA) and 5-HIAA according to the method of Mohanakumar et al. (1994). The HPLC system was equipped with a Universal injector, electrochemical detector (460, Waters) and an ion-pair, Ultrasphere RP analytical column (4.6 cm $\times$ 25 cm) with 5 $\mu$m particle size (Beckman, Fullerton, CA). The mobile phase contained 8.65 mmol heptane sulfonic acid $^{14}$, 0.27 mmol EDTA $^{14}$, 13% (v/v) acetonitrile, 0.4–0.45% (v/v) triethylamine and 0.20–0.25% (v/v) phosphoric acid. The flow rate was 0.7 ml min$^{-1}$ and the electrodetection was performed at 0.74 V. The data were collected and integrated in a Waters 745B data module. The peak heights were measured and compared with authentic samples. Results are presented as pmole mg$^{-1}$ fresh tissue and are uncorrected values.

Table 1. Effects of chronic treatment of quinalphos and oestradiol alone or in combination for 15 days on the mass of sex organs and on the serum concentrations of gonadotrophins (LH, FSH, prolactin) and testosterone in adult male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paired testes (g)</th>
<th>Seminal vesicle (mg)</th>
<th>Prostate (mg)</th>
<th>Pituitary (mg)</th>
<th>LH (ng ml$^{-1}$)</th>
<th>FSH (ng ml$^{-1}$)</th>
<th>Prolactin (ng ml$^{-1}$)</th>
<th>Testosterone (ng ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1.47 ± 0.04</td>
<td>230.09 ± 46.18</td>
<td>95.45 ± 17.0</td>
<td>1.96 ± 0.13</td>
<td>0.92 ± 0.11</td>
<td>5.44 ± 0.16</td>
<td>3.87 ± 0.42</td>
<td>1.28 ± 0.21</td>
</tr>
<tr>
<td>Quinalphos (7 mg per kg per day)</td>
<td>1.26 ± 0.02</td>
<td>394.64 ± 13.13**</td>
<td>134.96 ± 3.97**</td>
<td>2.11 ± 0.06</td>
<td>1.61 ± 0.19*</td>
<td>7.53 ± 0.68*</td>
<td>6.73 ± 0.10*</td>
<td>5.62 ± 0.62**</td>
</tr>
<tr>
<td>Oestradiol (50 $\mu$g per rat per day)</td>
<td>0.94 ± 0.11**</td>
<td>75.84 ± 3.30**</td>
<td>25.58 ± 2.55**</td>
<td>6.05 ± 0.24**</td>
<td>ND</td>
<td>ND</td>
<td>21.95 ± 1.80*</td>
<td>ND</td>
</tr>
<tr>
<td>Pesticide + oestradiol</td>
<td>0.79 ± 0.08**</td>
<td>56.92 ± 6.49**</td>
<td>22.66 ± 2.31**</td>
<td>6.12 ± 0.31**</td>
<td>ND</td>
<td>ND</td>
<td>11.76 ± 0.58**</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; *$P \leq 0.05$; **$P \leq 0.01$ compared with the vehicle-treated group (ANOVA and Duncan’s multiple-range test).

ND: not detectable.

Results

Gross morphology

Oral administration of the highest dose of quinalphos (14 mg kg$^{-1}$) produced signs of cholinergic hyperactivity such as salivation and muscular tremors after 4–5 h of treatment. The signs of organophosphate poisoning were perceptible to moderate, depending on the dose administered. Body weight and testicular mass of rats exposed to low doses (7 mg kg$^{-1}$) of pesticide did not show any significant change, whereas oestradiol alone or in combination with pesticide (low dose) caused a significant inhibition in testis mass (Table 1). Pesticide at doses of 10 and 14 mg kg$^{-1}$ caused significant decreases in paired testicular mass of 1.18 ± 0.05 and 1.02 ± 0.08 g per 100 g body weight, respectively, compared with the control (1.47 ± 0.04 g per 100 g body weight). However, the mass of secondary sex organs was not significantly affected.
organs in pesticide-treated rats increased significantly (Fig. 1), whereas pesticide-induced increases in the mass of the secondary sex organs were counteracted by oestradiol. Pituitary mass remained almost unchanged in pesticide-treated rats and showed a threefold increase after oestradiol treatment (Table 1).

**Spermatogenesis**

Testicular sections showed normal spermatogenesis in control groups (Fig. 2a). However, after pesticide treatment, different degrees of degenerative changes in spermatogenesis were observed. The damage that occurred was variable and the tubules were not affected uniformly. At the lowest dose of pesticide (7 mg kg⁻¹), spermatogenesis was qualitatively normal, although some tubules contained spherical vacuoles (Fig. 2b) and showed disruption in normal epithelial organization (Fig. 2c). Moreover, tubular damage increased with the increasing dose of pesticide. The absence of multiple steps of germ cell development resulted in the ability to distinguish complete stage profiles normally associated with the seminiferous epithelial cycle. At the highest dose of pesticide (14 mg kg⁻¹), seminiferous tubules were markedly reduced in cross-sectional area due to collapse of the seminiferous epithelium, which showed a variety of cellular damage ranging from moderate reduction in the number of germ cells to elimination of all cells from the tubules (Fig. 2d). There were granular depositions and vacuoles in certain regions in interstitial spaces. In the most severely damaged tubules, germ cells were not detectable; several multinucleated bodies and cells, that is giant cells (Fig. 2d), were frequently found together with large vacuoles. In oestradiol-treated rats, spermatogenesis was arrested at the primary spermatocyte stage in some tubules (Fig. 2e). In both pesticide- and oestradiol-treated rats, seminiferous tubules were almost devoid of cells except for the spermatogonia, Sertoli cells and a few giant cells (Fig. 2f).

**Changes in AChE and ACE activity**

AChE activity in pesticide-treated animals was inhibited in the hypothalamus, pituitary (Fig. 3) and the testis (Fig. 4) in a dose-dependent manner. Oestradiol treatment inhibited AChE activity in the pituitary much more than the pesticide (Table 2). In organophosphate-treated rats, ACE activity

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**Table 2.** Effects of chronic treatment of quinalphos and oestradiol alone or in combination for 15 days on the AChE activity in the testis, pituitary and hypothalamus of adult male rats killed 1 day after the last dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testis</th>
<th>Hypothalamus</th>
<th>Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>5.00 ± 0.43</td>
<td>40.12 ± 3.85</td>
<td>113.43 ± 13.00</td>
</tr>
<tr>
<td>Quinalphos (7 mg per kg per day)</td>
<td>5.25 ± 0.23</td>
<td>21.78 ± 2.40ᵇ</td>
<td>28.09 ± 1.48ᵇ</td>
</tr>
<tr>
<td>Oestradiol (50 µg per rat per day)</td>
<td>5.56 ± 0.21</td>
<td>31.72 ± 3.51</td>
<td>3.56 ± 0.74ᵇ</td>
</tr>
<tr>
<td>Pesticide + oestradiol</td>
<td>6.25 ± 0.55</td>
<td>36.04 ± 2.69</td>
<td>4.88 ± 0.23ᵇ</td>
</tr>
</tbody>
</table>

¹P ≤ 0.05, ²P ≤ 0.001 compared with the vehicle-treated group (ANOVA and Duncan’s multiple-range test). ***, indicates significant differences at P ≤ 0.01.
increased significantly in the testis (Fig. 4) and decreased in the pituitary (Table 3). Oestradiol treatment did not affect ACE activity in the pituitary. The inhibitory effect of organophosphate on ACE was blocked by oestradiol (Table 3).

**Effects on LH, FSH, prolactin and testosterone concentrations**

Quinalphos treatment enhanced serum hormone concentrations in a dose-dependent manner (Figs 1 and 5).
An approximately fourfold increase in serum gonadotrophins (LH and prolactin) was observed at the highest dose (Fig. 5). Serum FSH concentrations were significantly increased after pesticide treatment (Table 3). However, in the groups treated with oestradiol alone or in combination with pesticide, serum LH, FSH and testosterone remained lower than in the control group (Tables 1 and 3). Serum prolactin was increased in all the experimental groups.

Changes in neurotransmitter metabolism in hypothalamus

Chronic administration of either pesticide or oestradiol did not cause significant changes in dopamine, noradrenaline, 5-HT and 5-HIAA in the hypothalamus. However, a combination of quinalphos and oestradiol resulted in significant inhibition of dopamine and 5-HIAA and increases in 5-HT and noradrenaline (Table 4 and Fig. 6). The inhibitory effect on dopamine and the stimulatory effect on noradrenaline by oestradiol alone were potentiated by the pesticide treatment (Fig. 6). An analysis of the ratio of noradrenaline : dopamine indicated an interdependency of these two neurotransmitters in the hypothalamus (data not shown). The turnover of dopamine, that is, the ratio of metabolites (DOPAC + HVA) to the precursor (dopamine) was increased after pesticide or oestradiol treatment (see Table 5). However, treatment with a combination of pesticide and oestradiol resulted in a synergistic effect on the turnover of the amine. Turnover of 5-HT was inhibited in the treatment groups (Table 4).

Discussion

This study is the first to show that exposure of adult male rats to sub-lethal dosages of an organophosphate pesticide, quinalphos, can alter reproductive function by increasing the...
variable spermatogenetic disruption (Rivier, 1986) as LH has a central role in initiating and sustaining high concentrations of circulating LH (Kerr and Sharpe, 1986). Dense aggregations of extracellular material may be due to degeneration of Sertoli cells and germ cells, and the presence of germ cells (Chapin and Lamb, 1984). Administration of organophosphate in adult male rats inhibited activity of AChE in the hypothalamus, pituitary and testis and decrease in testicular mass was consistent with elimination of serum gonadotrophins. Paired testicular mass, a valuable index of reproductive toxicity in male animals (Amann, et al., 1980; Lefebvre, 1979). Histological data indicate that organophosphate treatment can focally inhibit spermatogenesis and, in certain areas of the seminiferous tubules, destroy all the cells of the seminiferous epithelium. Similar effects on the seminiferous epithelium were observed after treatment with LH alone or in combination with LHRH agonist (Sharpe et al., 1983; Kerr and Desjardins, 1982). Thus, high LH concentrations stimulate the Leydig cells to release large amounts of testosterone, which causes abnormal growth of the accessory sex organs. Secondly, organophosphate pesticides may act directly on Leydig cells to stimulate testosterone synthesis, as observed in the present studies. Thirdly, increased serum prolactin regulates the population of LH receptors, which in turn facilitates LH-stimulated testosterone production (Morris and Saxena, 1980). Direct correlation of serum prolactin with testicular LH receptor concentrations are well established (Chowdhury et al., 1983).

Investigation of ACE activity in organophosphate-treated rats revealed that ACE has two opposite actions on the pituitary and testis. Inhibition of pituitary ACE activity may cause inhibition of pituitary angiotensin II, which in turn increases the secretion of prolactin from the anterior pituitary, as administration of angiotensin II results in a decrease in the secretion of growth hormone and prolactin by the direct action of angiotensin II at the anterior pituitary (Steel et al., 1981). Alternatively, increased testicular ACE activity may be due to increased production of testosterone, which is also essential for the development and maintenance of testicular ACE (Velletri et al., 1985).

Oestradiol was administered to pesticide-treated animals mass of accessory sex organs and increasing concentrations of serum gonadotrophins. Paired testicular mass, a valuable index of reproductive toxicity in male animals (Amann, 1982), increased with increasing dose of pesticide and this decrease in testicular mass was consistent with elimination of germ cells (Chapin and Lamb, 1984). Administration of organophosphate in adult male rats inhibited activity of AChE in the hypothalamus, pituitary and testis and increased serum testosterone concentrations. ACE activity was decreased in the pituitary and increased in the testis of the pesticide-treated rats.

Histological data indicate that organophosphate treatment can focally inhibit spermatogenesis and, in certain areas of the seminiferous tubules, destroy all the cells of the seminiferous epithelium. Similar effects on the seminiferous epithelium were observed after treatment with LH alone or in combination with LHRH agonist (Sharpe et al., 1983; Kerr and Sharpe, 1986). Focal degeneration of the seminiferous epithelium, as observed in the present study, represents a very localized toxicological effect from which a proportion of seminiferous tubules fail to recover their spermatogenetic function (Pelletier et al., 1980; Lefebvre et al., 1984). Degeneration of Sertoli cells and germ cells, and the presence of dense aggregations of extracellular material may be due to high concentrations of circulating LH (Kerr and Sharpe, 1986) as LH has a central role in initiating and sustaining variable spermatogentic disruption (Rivier et al., 1979). Increases in mean serum testosterone concentrations in organophosphate-treated rats may be due to more than one mechanism. Firstly, increases in serum testosterone concentrations may be explained by the fact that the treatment causes an increase in serum LH concentrations. In male rats, circulating LH is responsible for maintaining normal increased serum testosterone concentrations (Ellis and Desjardins, 1982). Thus, high LH concentrations stimulate the Leydig cells to release large amounts of testosterone, which causes abnormal growth of the accessory sex organs. Secondly, organophosphate pesticides may act directly on Leydig cells to stimulate testosterone synthesis, as observed in the present studies. Thirdly, increased serum prolactin regulates the population of LH receptors, which in turn facilitates LH-stimulated testosterone production (Morris and Saxena, 1980). Direct correlation of serum prolactin with testicular LH receptor concentrations are well established (Chowdhury et al., 1983).

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**Table 4.** Effects of chronic treatment of quinalphos and oestradiol alone or in combination for 15 days on the content and turnover of 5-HT in the pituitary and hypothalamus of adult male rats killed 1 day after the last dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5-HT Hypothalamus</th>
<th>5-HT Pituitary</th>
<th>5-HIAA Hypothalamus</th>
<th>5-HIAA Pituitary</th>
<th>5-HIAA:5-HT Hypothalamus</th>
<th>5-HIAA:5-HT Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>0.61 ± 0.08</td>
<td>1.58 ± 0.23</td>
<td>5.99 ± 0.50</td>
<td>0.62 ± 0.08</td>
<td>10.22 ± 0.96</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Quinalphos (7 mg per kg per day)</td>
<td>0.79 ± 0.07</td>
<td>2.46 ± 0.16a</td>
<td>7.23 ± 0.40</td>
<td>0.84 ± 0.06</td>
<td>9.50 ± 1.21</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Oestradiol (50 µg per rat per day)</td>
<td>0.85 ± 0.19</td>
<td>2.12 ± 0.20</td>
<td>7.49 ± 0.61</td>
<td>0.77 ± 0.06</td>
<td>8.87 ± 1.40</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Pesticide + oestradiol</td>
<td>1.07 ± 0.01</td>
<td>2.75 ± 0.22b</td>
<td>3.89 ± 0.44*</td>
<td>1.06 ± 0.09b</td>
<td>3.64 ± 0.56b</td>
<td>0.35 ± 0.02</td>
</tr>
</tbody>
</table>

5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.

*P ≤ 0.05; **P ≤ 0.01 compared with the vehicle-treated group (ANOVA and Duncan’s multiple-range test).

**Table 5.** Effects of chronic treatment of quinalphos and oestradiol alone or in combination for 15 days on the metabolites of dopamine and its turnover in the pituitary and hypothalamus of adult male rats killed 1 day after the last dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HVA Hypothalamus</th>
<th>HVA Pituitary</th>
<th>DOPAC Hypothalamus</th>
<th>DOPAC Pituitary</th>
<th>Dopamine turnover* Hypothalamus</th>
<th>Dopamine turnover* Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>0.55 ± 0.02</td>
<td>ND</td>
<td>1.22 ± 0.10</td>
<td>1.46 ± 0.09</td>
<td>0.51 ± 0.05</td>
<td>0.92 ± 0.07</td>
</tr>
<tr>
<td>Quinalphos (7 mg per kg per day)</td>
<td>0.76 ± 0.07a</td>
<td>ND</td>
<td>1.62 ± 0.15</td>
<td>2.26 ± 0.23a</td>
<td>0.58 ± 0.03</td>
<td>1.40 ± 0.15</td>
</tr>
<tr>
<td>Oestradiol (50 µg per rat per day)</td>
<td>0.54 ± 0.06</td>
<td>ND</td>
<td>1.54 ± 0.18</td>
<td>1.84 ± 0.05</td>
<td>0.68 ± 0.09</td>
<td>4.71 ± 0.17</td>
</tr>
<tr>
<td>Pesticide + oestradiol</td>
<td>0.50 ± 0.06</td>
<td>ND</td>
<td>1.35 ± 0.12</td>
<td>3.02 ± 0.32b</td>
<td>0.78 ± 0.01b</td>
<td>7.19 ± 1.24b</td>
</tr>
</tbody>
</table>

HVA: homovanillic acid; DOPAC: 2,4-dihydroxyphenyl acetic acid.

*P ≤ 0.05; **P ≤ 0.01 compared with the vehicle-treated group (ANOVA and Duncan’s multiple-range test).

Dopamine turnover in the pituitary is DOPAC:dopamine and in the hypothalamus is (DOPAC + HVA):dopamine.

ND: not detectable.
to determine whether the steroidal feed-back mechanism was operative in these animals. The results of the present study indicate that oestradiol counteracted the observed pesticide effects on gonadotrophins. This indicates that the gonado–pituitary function in pesticide toxicity is intact.

An unexpected finding was the organ-specific potential inhibitory action of oestradiol on AChE activity in the pituitary. This indicates a direct and hitherto unknown action of a steroid on AChE. The relevance of such an effect of oestradiol remains to be determined. The results of the present study indicate that organophosphates have a direct effect on AChE, resulting in alterations in pituitary gonadotrophins. The observed effect of oestrogen on pituitary AChE activity indicates that the organophosphate could influence testicular function directly through its effect on pituitary AChE.

In the present study, the concentrations of different neurotransmitters were evaluated to determine the mechanisms underlying the effects of the pesticide. Although pesticide administration at the lowest dose caused significant changes in LH, prolactin and testosterone, there was no significant effect on the concentrations of noradrenaline, dopamine and 5-HT in the hypothalamus or pituitary. Similarly, oestradiol treatment did not alter the concentration of neurotransmitters in these regions and caused a significant decrease in organophosphate-induced increases in hormone concentrations. This may be due to the fact that the neurotransmitters maintain a homeostatic plateau after a constant toxic insult in the brain. This plateau is achieved through changes in the metabolism of the neurotransmitters or in the density or sensitivity of their receptors. Acute organophosphate administration in laboratory animals alters a number of other neurotransmitters in addition to AChE in various parts of the brain (Ali et al., 1979, 1980; Potter et al., 1985; Robinson and Hambrecht, 1988). In the present study, the acute effects of quinalphos were not analysed. The results of the present study are similar to the findings of Potter et al. (1985) who reported an increased turnover of dopamine after chronic pesticide treatment. In the present study the turnover of dopamine was increased and of 5-HT was decreased in the pituitary and hypothalamus after chronic administration of the pesticide, and this indicates a direct effect of quinalphos. Similarly, chronic administration of di-isopropyl fluorophosphate, another organophosphate, upregulates dopamine, ACh and gamma-aminobutyric acid (GABA) receptors in the striatum (Sivum et al., 1983). The lack of any significant effect on the content of biogenic neurotransmitters, alternatively, reflects the lack of any behavioural dysfunction after the low doses of quinalphos since severe fluctuations in the neurotransmitters should have resulted in behavioural abnormalities.

The findings of the present study show that fluctuations in the neurotransmitters caused by treatment with pesticide and oestradiol alone or in combination do not reflect on the status of circulatory LH or FSH. This may indicate that changes in the metabolism of neurotransmitters are independent of the effects on LH or FSH, or vice versa. Increases in noradrenaline or 5-HT and decreases in dopamine in the hypothalamus and pituitary indicate that the effects of the pesticide can be potentiated in an environment in which steroid concentrations are high. Although there are reports on the direct effects of 5-HT and noradrenaline on the release of prolactin (Clemens et al., 1978; Pilote and Porter, 1981) and LHRR (Ojeda et al., 1982) from the pituitary, respectively, independent of any influence of dopamine in male rats, it is difficult to assess the cause–effect relationship from the results of the present study.

In conclusion, it may be postulated that the initial effects of the pesticide are the result of increases in LH and testosterone concentrations, which affect spermatogenesis. Pesticide-induced inhibition of AChE in turn might increase concentrations of ACh in the pituitary and hypothalamus,
which in the complex circuitry of neuroendocrine regulation can invariably affect a secondary transmitter, especially dopamine or 5-HT (see Corrodi et al., 1967; Butcher, 1979; Robinson, 1983; Bradford, 1986).

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Fig. 6. Effect of quinalphos (7 mg kg⁻¹) and oestradiol (50 µg per rat) alone or in combination on noradrenaline and dopamine contents in the hypothalamus (a,c) and pituitary (b,d) of adult male rats after 15 days. Values are mean ± SEM. Significant differences are indicated by asterisks (*P ≤ 0.05, **P ≤ 0.01).
Prolonged cholinergic stimulation can cause low levels of DA resulting from high DA turnover. Life Science 6:225–232.


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