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Synergistic Effects of Aroclor® 1254 and Mirex on the Semen Characteristics of American Kestrels

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Abstract. Semen characteristics of captive American Kestrels (*Falco sparverius*) fed 8 ppm mirex or 33 ppm Aroclor® 1254, singly or in combination, were compared to a control group. Aroclor® 1254 produced a decline in sperm concentration but no compensatory increase in semen volume, resulting in a 22–27% decrease in sperm numbers per ejaculate. Mirex produced a marked decline in sperm concentration with a slight compensatory increase in semen volume resulting in a 70% decrease in sperm numbers. The combination of Aroclor® 1254 and mirex, at the same concentrations as fed singly, decreased sperm concentration to the same level as the Aroclor® alone with a 73% increase in semen volume, resulting in sperm numbers which did not differ from those of the controls. No effect on sperm motility was observed for any contaminant. Temporal patterns in semen characteristics differed. There was a highly significant interaction effect between Aroclor® 1254 and mirex. The excretion of mirex was increased in the presence of Aroclor® 1254, and the combination diet reduced the relative concentration of Aroclor® in the testes. The testicular mass of the PCBs + mirex group was increased. The results suggest that migratory flesh-eating birds feeding on a PCB- or mirex-contaminated food chain could consume enough toxicant to alter their semen quality in that breeding season, which, when coupled with altered courtship, could reduce the fertility of eggs and the reproductive fitness of the individual.

The impact of environmental contaminants on the male reproductive system is poorly documented (Steinberger 1981). Chemically-induced male infertility related to occupation was reported for a group of factory workers (Whorton *et al.* 1977) which manufactured 1,2-dibromo-3-chloropropane and in agricultural workers who applied this nematocide (Takahashi *et al.* 1981). Circumstantial evidence suggests that the unknown etiology of nearly one-half of the cases of human male infertility might be attributed to chronic exposures to various environmental and occupational chemicals (Dixon *et al.* 1979).

Although the impact of several persistent organochlorine environmental contaminants on avian reproduction have been studied in domesticated and wild bird species, the relative role of the sexes in any abnormal reproductive outcomes has seldom been determined. In addition, few studies have assessed the impact of combinations of contaminants relative to the impact of the individual components. Food chains are seldom contaminated with a single pollutant and seldom, if ever, is only one sex exposed.

Polychlorinated biphenyls (PCBs) and mirex (dodecachloro octahydro - 1,3 - 4 metheno -2*H* - cyclobuta[c,d]pentaline) are environmentally stable and slowly metabolized xenobiotics which contaminate aquatic and terrestrial food chains (Peakall 1972; Borthwick *et al.* 1973; Norstrom *et al.* 1978). Herring Gulls (*Larus argentatus*) breeding on Lake Ontario are contaminated with both pollutants (Norstrom *et al.* 1978). This population suffered poor

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reproductive success in the early 1970s, the period of peak contamination, while those in the Great Lakes not substantially contaminated by mirex did not (Gilman *et al.* 1977). Numerous studies have been undertaken to assess the effects of these contaminants individually on captive wild and domesticated bird species but none have considered them in combination. Studies of the effects of PCBs on semen quality of domesticated fowl have been published (Lillie *et al.* 1974; Ahmed *et al.* 1978) but we know of no similar studies with mirex.

The availability of a captive breeding colony of American Kestrels (*Falco sparverius*) at McGill University (Bird and Rehder 1981; Bird 1982) and the development of a massage technique for semen collection in this species (Bird *et al.* 1976) provided the opportunity to investigate the impact of PCB and mirex administered singly and in combination, via the diet, on the semen quality of a wild predatory bird species.

Methods

Experimental Subjects and Housing

In March 1977, yearling male kestrels bred at the Macdonald Raptor Research Center were randomly assigned to either the control or PCBs treatment groups each containing 10 individuals. In 1978, yearling males were assigned randomly to the control group (n = 10), PCBs group (n = 10), or one of two additional groups: mirex (n = 5) and PCBs + mirex (n = 5). The birds were tethered on wooden perches and housed in groups of five in well-ventilated rooms either with natural lighting or an artificial photoperiod simulating natural day length changes.

Diets

Mirex and furan-free Aroclor® 1254 (Bowes *et al.* 1973) were dissolved in peanut oil in concentrations of 2.5 mg/ml and 10 mg/ml, respectively; 0.1 ml was injected into the breast muscle of day-old cockerels. Day-old cockerels similarly injected with peanut oil constituted the control diet. These dosages represent 8 ppm mirex or 33 ppm PCBs in the diet on a wet weight basis of 2.3–2.5 mg/kg and 9–10 mg/kg body weight/day, respectively. Vitamin and calcium supplements (Coopérative Fédérée de Québec, Montréal) were smudged on the abdomens of the cockerels three times per week. Each kestrel received one cockerel per day but in very cold weather an extra untreated cockerel was provided.

Semen Collection and Analysis

Semen was collected from every kestrel once each four days by the modified abdominal massage technique (Bird *et al.* 1976). However, on May 7, 1978, four males from the control and two from the PCBs group producing no semen to this date were eliminated from the study and semen was subsequently collected from the remaining birds every second day. The collection period lasted 69 days in 1977 (March 25 to June 2) and 62 days in 1978 (March 24 to May 25). Within one hour of collection, each semen sample was analyzed for the following characteristics; semen volume, sperm concentration, sperm count per ejaculate, and sperm motility (Bird and Laguë 1977). At the termination of the 1978 semen collection period, the birds were sacrificed with their testes removed and weighed to the nearest 0.1 mg.

Residue Analysis

At the termination of the 1978 collection period, the livers, testes, and left side of the pectoral muscle from each of the individuals were pooled by group and tissue, and submitted to the Ontario Research Foundation, Sheridan Park, Mississauga, Ontario, for organochlorine analysis (Reynolds and Cooper 1975).

Statistical Analysis

One-way ANOVA and linear regression analysis (SAS) were performed on the 1977 data, whereas the Factorial Analysis using the general linear model (SAS) was used in 1978 for semen volume, sperm concentration, and sperm numbers per ejaculate. Linear regression analysis of the 1978 data was performed using the SYSREG procedure of SAS (Barr *et al.* 1976). Since sperm motility was measured on an ordinal scale and as sample sizes for the testicular mass were small, the nonparametric Mann-Whitney U or Kruskal-Wallace one-way ANOVA were used (Siegel 1956).

Results

Quantitative Differences in Semen Characteristics

The various measures of semen quality are summarized in Table 1. Since the contaminants altered the parameters in different ways, we have chosen to treat the results from each contaminated diet separately.

In 1977, Aroclor® 1254 increased semen volume by 10.4% ($P > 0.05$), and produced a decline ($P < 0.005$) in sperm concentration of 22.6%. The result was a 22.0% decrease ($P > 0.10$) in sperm numbers per ejaculate. Sperm motility was not affected. Aroclor® 1254 did not increase semen volume in 1978. However, it did produce a decline ($P < 0.01$) in sperm concentration of 21.5%, resulting in a 26.8% decrease ($P < 0.025$) in sperm numbers per ejaculate. Sperm motility was not affected. Mirex increased ($P > 0.10$) semen volume by 8.4% but markedly reduced sperm concentration by 40.3% ($P < 0.0005$). The combined result was a 70.5% decrease ($P < 0.0015$) in sperm numbers per ejaculate. Sperm motility was decreased by 9.6% ($P > 0.10$). In contrast to the effects of the individual components, the combination increased semen volume by 73.3% ($P < 0.003$) and only decreased the sperm

Table 1. Semen characteristics and their temporal trends in male American Kestrels fed organochlorine-contaminated diets

Diet group	Year	Semen volume in mm^3	Sperm concentration $\times 10^3$ per mm^3	Sperm per ejaculate $\times 10^3$	Motility score
Control	1977	7.58 ± 0.51 (97)[a] Y = −0.0227X + 7.700[b]	38.00 ± 2.63 (91) Y = 2.0880X + 20.8040	335.4 ± 33.8 (91) Y = 1.4144X + 20.7160	3.80 ± 0.19 (91)
Control	1978	7.27 ± 0.84 (51) Y = 0.0941X + 6.1548	40.70 ± 2.56 (41) Y = 1.4546X + 24.5238	327.6 ± 50.2 (41) Y = 1.5709X + 15.2880	3.54 ± 0.14 (41)
PCB	1977	8.37 ± 0.72 (90) Y = −0.4393X + 12.2720†	29.42 ± 2.16 (90)* Y = 0.8835X + 20.8524†	261.6 ± 39.5 (90) Y = −1.0521X + 36.2206†	3.80 ± 0.26 (90)
PCB	1978	7.34 ± 0.48 (103) Y = 0.01301X + 5.7731	31.97 ± 2.09 (100)* Y = −0.8646X + 42.1292† ††	233.8 ± 20.9 (100) * ** Y = −0.3973X + 28.1710† ††	3.60 ± 0.31 (100)
Mirex	1978	7.88 ± 0.67 (63) Y = 0.0833X + 6.7704	14.45 ± 1.86 (60)* ** Y = −0.3130X + 17.5821† ††	96.8 ± 11.3 (60)* ** Y = −0.1100X + 10.7787† ††	3.20 ± 0.13 (60)
PCBs + Mirex	1978	12.89 ± 1.38 (55)* Y = 0.1242X + 14.0484†	32.45 ± 3.25 (41)* Y = −2.0792X + 52.0747†	335.8 ± 56.3 (41) Y = −1.9478X + 52.0.74	3.81 ± 0.11 (41)

[a] Mean ± S.D. (n)
[b] equation for regression of semen characteristics against time
* = contaminant group significantly different from respective control ($P \leq 0.05$)
** = PCB or mirex group significantly different from PCBs + mirex combination ($P \leq 0.05$)
† = slope of contaminant group significantly different from respective control ($P \leq 0.05$)
†† = slope of PCBs or mirex group significantly different from PCBs + mirex combination ($P \leq 0.05$)

concentration by 20.3% ($P < 0.0001$) roughly equivalent to the reduction produced by Aroclor® alone. The markedly increased semen volume and moderate decline in sperm concentration resulted in sperm numbers per ejaculate which were almost identical to those of the control group. Sperm motility was increased by 7.6%. Factorial analysis revealed a highly significant interaction effect ($P < 0.0001$) between the Aroclor® and mirex.

Temporal Differences in Semen Characteristics

Semen volume: In 1977, semen volume for the control group varied little with time (Figure 1). The PCBs group showed a significant decrease with time with much larger initial volumes than the control group. In 1978, the control, PCBs and mirex groups all exhibited slight increases in volume over time (Figure 1). In contrast, the PCBs + mirex group showed a definite decrease in volume with time.

Sperm concentrations: In 1977, sperm concentrations of the control group increased with time (Figure 2), while those of the PCBs group increased at a much slower rate ($P < 0.025$). In 1978, the sperm concentration of the control group also increased with time (Figure 2), while those of the contaminated groups decreased, the decrease being most marked in the PCBs + mirex and PCBs groups. The sperm concentration of the mirex group remained at a relatively low level throughout the sampling period.

Sperm numbers per ejaculate: In 1977, sperm numbers per ejaculate of the control group increased over time (Figure 3), whereas those of the PCB group declined ($P < 0.05$). In 1978, the values for the control group also increased over time (Figure 3) in contrast to those of all the contaminated groups which declined at varying rates. The rate of decline in the PCBs + mirex group was markedly more rapid than that of the PCBs or mirex groups.

The PCBs + mirex group showed a similar temporal pattern for all parameters; a marked increase to very high peak levels between the fifth and tenth collection day followed by a marked decline.

Testicular Mass

The median testicular mass, at the termination of the experiment, for the control, PCBs, and mirex groups was 45 mg in each case in contrast with 60 mg in the PCBs + mirex group ($P = 0.075$, Mann-Whitney U test).

Tissue Distribution of Contaminant Residues

The residue content of the pooled livers, muscle and testes, expressed on a lipid weight basis of the

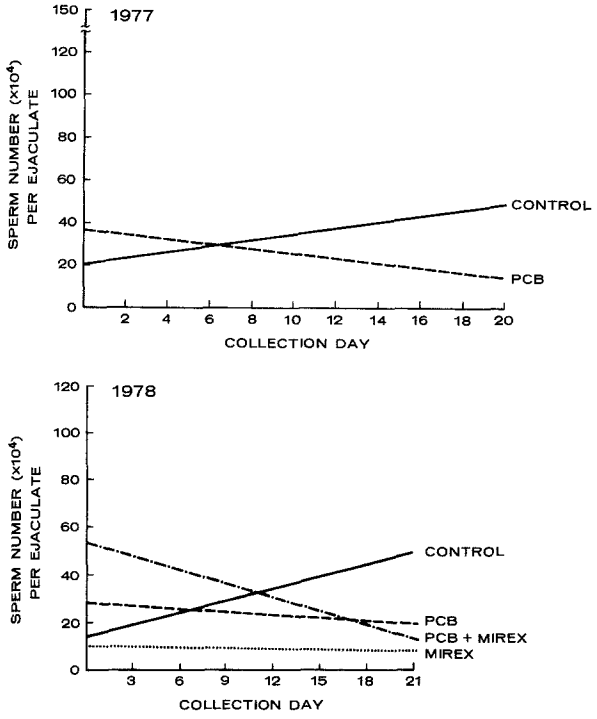

Fig. 1. Linear regression analysis of temporal variation in volume of semen ejaculates of male American Kestrels fed organochlorine-contaminated cockerels. Equations and number of observations upon which lines are based are given in Table 1

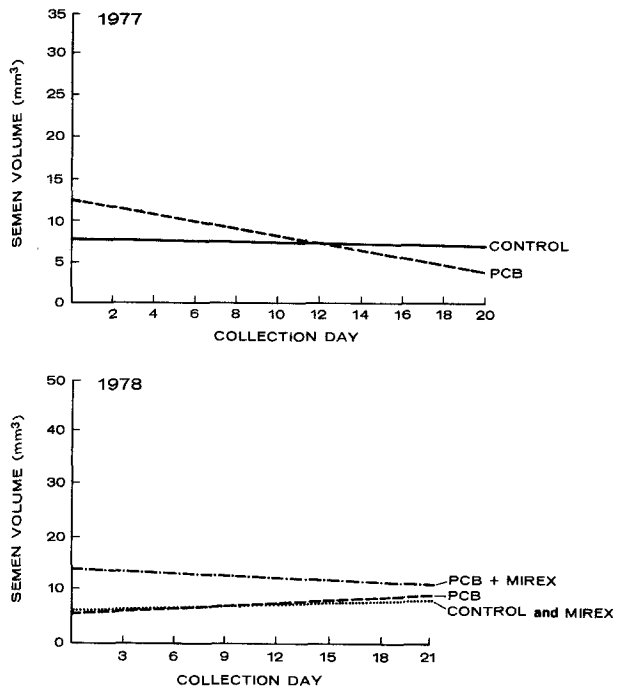

Fig. 2. Linear regression analysis of temporal variation in the sperm concentration of semen of male American Kestrels fed organochlorine-contaminated cockerels. Equations and number of observations upon which lines are based are given in Table 1

1978 study are summarized in Table 2. The liver had the lowest content of DDE, PCBs, and mirex regardless of treatment consistent with its role in metabolism and excretion of xenobiotics. The testes contained the highest PCBs and DDE concentrations in the control, PCBs, and mirex groups. There was a decrease in mirex accumulated in all the tissues of the PCBs + mirex group.

Discussion

This is one of four published studies of the effects of polyhalogenated hydrocarbons on the semen quality of birds and is the first to report the effects upon a wild carnivorous species. The results cannot be compared directly to those of other investigators who have used histological, biochemical, or fertility measures. Sperm concentration and sperm numbers per ejaculate generally increase during the collection period due to conditioning and facilitation (Bird, unpubl.) as seen in the controls in this study. Contaminants reduced or reversed this response.

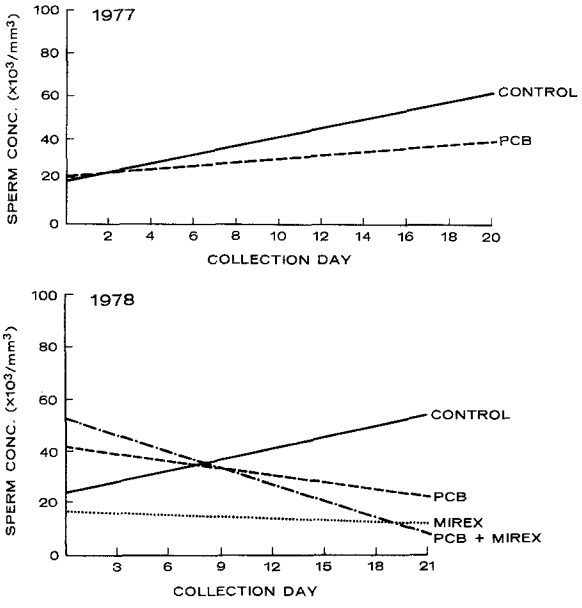

Fig. 3. Linear regression analysis of temporal variation in numbers of sperm per ejaculate from male American Kestrels fed organochlorine-contaminated cockerels. Equations and number of observations upon which lines are based are given in Table 1

Table 2. Residue content of pooled tissues from male American Kestrels fed organochlorine-contaminated diets, 1978

Diet	Tissue (n)	Percent lipid	Residue in ppm, lipid weight	
			Aroclor® 1254	Mirex
Control	Liver (5)	4.3	ND[a]	ND
	Muscle (5)	3.2	0.41	ND
	Testes (10)	10.5	1.03	ND
PCBs	Liver (9)	3.9	91.6 (1)	ND
	Muscle (9)	3.5	107.3 (1.17)	ND
	Testes (18)	7.0	127.9 (1.40)	ND
Mirex	Liver (5)	4.2	ND	38.2 (1)
	Muscle (5)	3.6	ND	56.0 (1.47)
	Testes (10)	9.1	1.11	49.7 (1.30)
PCBs + mirex	Liver (5)	5.1	75.2 (1)	23.3 (1)
	Muscle (5)	3.9	123.6 (1.64)	39.7 (1.70)
	Testes (10)	6.9	117.1 (1.56)	33.5 (1.44)

[a] = not detected

Incorporation of Aroclor® 1254 into the diet of male American Kestrels resulted in decreased sperm concentration. Ahmed *et al.* (1978) found that 10 to 40 pm of this contaminant decreased semen volume, sperm concentration, and testes weight over a 40-week period in Leghorn cockerels. Aroclor® 1248 did not effect semen quality or testes weight of Leghorn cockerels when fed at 20 ppm for 8 weeks (Lillie *et al.* 1974). Ahmed *et al.* (1978) fed 25 and 50 ppm of the highly toxic pesticide dieldrin to Leghorn cockerels for 20 weeks without producing changes in semen quality. Similarly, Arscott *et al.* (1972) found no effect of DDE and DDT on semen quality of Leghorn cockerels, although Albert (1962) reduced sperm production with massive doses of DDT. Biessmann (1982) found Clophen® A60, another PCB mixture, decreased testicular weight, nuclear volume of the Leydig cells and the percentage of seminiferous epithelium of male Japanese Quail (*Coturnix c. japonica*). Platonow and Funnel (1971) observed reduced comb growth and testicular weight in Aroclor® 1254-dosed Leghorn cockerels. Aroclor® 1254 has also been shown to reduce testicular weights and/or alter testicular histology, or endocrine function in mice (Sanders and Kirkpatrick 1975) and fish (Freeman and Idler 1975; Freeman *et al.* 1982).

Dietary mirex markedly reduced sperm concentration of kestrels. We know of no other studies of the impact of this contaminant on semen quality. Incorporation of kepone (1,1a,3,3a,4,5,5,5a, - 5b,6 - decachloro octahydro - 1,3,4 - metheno - 2*H* - cyclobuta[c,d]pentalene - 2 - one) in the diet of male Japanese Quail increased testicular weight, increased tubule diameter, and increased numbers of interstitial cells but did not alter the pattern of spermatogenesis (McFarland and Lacy 1969). In contrast, kepone produced testicular atrophy in rats (Larson *et al.* 1979). Mirex reduces fertility and fecundity of mice (Ware and Good 1967; Shannon 1976) and decreases the incidence of female rats showing sperm in vaginal smears (Chu *et al.* 1981) but it is not possible to determine what role semen quality played in these changes.

The combination of Aroclor® 1254 and mirex resulted in alterations in semen quality which were very different from those of the individual components. Semen volume was markedly increased and sperm concentration was only reduced by an amount equivalent to that produced by PCB alone, roughly half that produced by mirex. The net effect was to reduce mean sperm numbers per ejaculate to the extent that they resembled those of the control group. However, this combination produced the greatest temporal decreases in semen volume, sperm concentration, and sperm numbers per ejaculate such that semen collected near the end of the study was of poor quality. No published studies are known of the effect of this combination on semen quality or reproduction in any species; our results suggest that the combination is synergistic.

Mehendale (1976) has shown that mirex reduces hepatobiliary excretion of PCBs in rats *in vitro*. Pepriell (1981) has shown that the combination of mirex and 3,4,5,3′,4′,5′-hexachlorobiphenyl (a component of Aroclor® 1254) results in the increased induction of both cytochromes P-450 and P-448 in mice. Hence, the combination of Aroclor® 1254 and mirex might be expected to result in increased excretion of mirex and decreased excretion and detoxification of PCBs. This was the case in our kestrels (for mirex only) and in free-living Lake Ontario Herring Gulls (*Larus argentatus*) (based on Norstrom *et al.* 1978) but not in Ring Doves (*Stepto-*

pelia risoria) (McArthur *et al.* 1983) or rats (Chu *et al.* 1980). The increased excretion of mirex by kestrels fed the combination could account for the decreased depression of sperm concentration when compared to those fed mirex alone. Aroclor® 1254 when fed to kestrels at the level of 0.5 and 5.0 ppm for five months increased the microsomal breakdown of the sex steroid estradiol by 13.6% and 60.5%, respectively (Lincer and Peakall 1970). The increased cytochrome P-448 and P-450 induction resulting from the combination of contaminants may have increased androgen metabolism and/or excretion and altered the normal feedback mechanisms resulting in increased FSH and LH secretion. This could increase semen volume and testicular mass.

PCB concentrations were higher in the testes than the liver or muscle (lipid weight basis) in all but the PCBs + mirex group, again suggesting that this combination alters the metabolism and/or excretion, and possibly storage of these lipophilic contaminants.

It is not known what the impact of the changes in semen quality observed in this study would be on fertility of free-living kestrels. However, Bird *et al.* (1976) have shown sperm count per ejaculate to be significantly and positively correlated with fertility. Ahmed *et al.* (1978) used artificial insemination techniques and were unable to show any impact of Aroclor® 1254-induced reductions in semen quality on the fertility of Leghorn cockerels. In wild population, both sexes are exposed to contaminants and successful reproduction requires both normal reproductive behavior and physiological function.

Copulation is frequent in birds possibly suggesting that sperm transfer is relatively inefficient. Lincer (1972) fed 10 ppm Aroclor® 1254 and/or 3 ppm DDE to captive kestrels and found that the combination delayed clutch initiation and decreased embryonation by 54% as compared with 24% and 36%, respectively, for DDE and PCBs alone, suggesting an additive response. Ring Doves fed a mixture of Aroclor® 1254, mirex, photomirex, and DDE had reduced plasma levels of sex steroids during the courtship period and clutch-initiation was delayed in a dose-related fashion (McArthur *et al.* 1983). The resulting courtship anomalies were apparently mediated through the female. In the same study, the high dosage diet, which contained 0.9 ppm mirex, 28 ppm Aroclor® 1254, 4.6 ppm DDE, and 0.3 ppm photomirex reduced hatching success and decreased incubation attentiveness and parental care. The impact of this mixture exceeded those reported for DDE or Aroclor® 1254 in Ring Doves.

Although no published studies are known of the impact of environmental contaminants on wild birds in which decreased fertility was established, this may represent inadequate methodology or lack of knowledge of the "normal" fertility for the species and population at risk. A migratory flesh-eating bird species, feeding in a PCB- or mirex-contaminated aquatic food chain, such as Lake Ontario Herring Gulls (Gilman *et al.* 1977, Norstrom *et al.* 1978) or Common Terns (*Sterna hirundo*) (Gilbertson 1974) could consume enough toxicant to alter semen quality in that breeding season. Such alterations in semen quality, when coupled with altered courtship behavior as reported by McArthur *et al.* (1983), could reduce the fertility of eggs and the reproductive fitness of the individual.

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