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TOXICOLOGICAL EFFECTS OF PROLONGED EXPOSURE TO γ-HEXACHLOROCYCLOHEXANE IN CHICKENS

(With 6 Tables and 2 Pictures)

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التأثيرات السامة لتعرض الدجاج لللندان لمدة طويلة

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تم في هذا البحث دراسة التأثيرات السامة التي تظهر على الدجاج البياض نتيجة تعرضه للمبيد الحشري اللندان. استخدم في هذه الدراسة عدد ثلاثين طائر قسمت إلى ثلاث مجموعات تعرضت المجموعة الأولى منها لتركيز يعادل ١٠ اجزء في المليون على الغذاء والثانية لتركيز يعادل ٥ جزء في المليون على الغذاء بينما استخدمت المجموعة الثالثة كضابط للتجربة. خلال التجربة تمت متابعة وزن الجسم الحي وحساب معدل النمو وكذا معدل إنتاج البيض ووزنه لمدة ١٨أسبوع وفي نهاية التجربة تم ذبح الطيور وأخذ عينات من دمائها لدراسة مدى التأثيرات التي حدثت نتيجة التعرض لمادة اللندان طوال هذه الفترة. لوحظ من خلال نتائج هذا البحث أن لللندان تأثيرات سمية على الطيور التي تعرضت له لمدة ١٨ أسبوع تمثلت في انخفاض معنوي جدا في كل من معدلات النمو وكمية الغذاء المستخدمة من قبل الطائر وبدا هذا التأثير مرتبط بالتركيز المستخدم حيث زاد هذا التأثير بزيادة التركيز المستخدم. سجل البحث أيضا تأثيرات ضارة على البروتين الكلي ومفرداته مما يعكس إمكانية تعرض هذه الطيور للعدوى بالأمراض أكثر من غيرها كما سجل زيادة في خميرة الكولين استراز في كل من المخ ومصل الدم في الطيور التي تعرضت لللندان ونقص كبير في تركيز كل من الكالسيوم والفوسفور والذي انعكس على عدم انتظام ترسيب الكالسيوم في قشرة البيض في المجموعات التي عوملت باللندان. يخلص البحث إلى ضرورة عدم تعوض الطيور إلى مركب اللندان ويوصى بعدم استخدامه في تطهير العنابر أو مقاومة الطفيليات الخارجية في الدجاج نظرا لخطورته على صحة الطائر.

SUMMARY

The toxic effects of γ-hexachlorocyclohexane (lindane) in chickens were studied. Thirty, three-month age, male and female Balady Behary chickens were divided into three groups (n=10, 5 males and 5 females).

The first group was exposed to 5 ppm and the second group with 10 ppm lindane in feed for 18 weeks; the third group was kept as control. Feed intake, body weight gain was recorded weekly while egg production as well as egg weight was recorded daily. After 18 weeks from treatments, all chickens were sacrificed by decapitation and free flowing blood was collected in clean vials for analysis. Liver, kidney, thyroid, thymus, spleen, heart, proventriculus, gizzard, intestine, ovary and oviduct, testes, and brain were dissected out and a post-mortem morphological examination was performed. Organs weights as well as organs-weight/body-weight ratio was calculated. Serum and brain acetylcholinesterase enzyme activity in both serum and brain, AST, ALT, total protein, albumin and globulin, creatinine, calcium and phosphorous in serum were also estimated. The results of this study revealed that, lindane had a significant adverse effects on body weight gain, feed intake, egg production and egg weight compared with birds given normal feed especially that group of birds treated with 10 ppm lindane. Significant changes in total protein; albumin, globulin and creatinine concentrations in the serum of treated birds were recorded. Also a highly significant increase in both AST, ALT, brain acetylcholinesterase and serum acetylcholinesterase activity in exposed male and female chickens was recorded. A highly significant decrease in calcium and phosphorous concentration in the serum of lindane-treated chickens as compared with control birds was also recorded.

Key words: *- Lindane; γ-HCH; Chickens; Organochlorine Insecticides.*

INTRODUCTION

γ-1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH) or lindane is one of the few organochlorine pesticides still used in agriculture and in veterinary medicine. Lindane is the common name for gamma-hexachlorocyclohexane, an insecticide formulated as a dust, in the form of aerosols, wettable powders, emulsions, granules and in tablets for fumigation (Aplada-Sarlis et al., 1994). Lindane has widespread environmental distribution resulting from its usage as an agricultural insecticide and also its use in human and veterinary medicine as an ectoparasiticide (Rasmussen, 1984; Fusia et al., 1987 and Criswell et al., 1995). It has been used for the protection against insects of wheat, cotton, corn and beet cultivation and in seed treatment (US EPA, 1988). It is a toxic organochlorine compound that can be hazardous to humans, animals, and birds as well as to the environment if incorrectly or carelessly handled. Lindane is degraded slowly in the environment, its field half life is usually well above 100 days depending on the specific site, the type of soil and the climate (Wauchope et al., 1992).

Studies have shown that lindane or other HCH isomers are nearly universal in human fat samples and tend to bioaccumulate with age (Adeshina and Todd, 1990; Robinson et al., 1990 and Szymczynski and Waliszewski, 1981). Although, lindane's primary biological response appears to be the production of hyperexcitability in either the mammalian or the insect central nervous system (Joy, 1982 and Woolley et al., 1985), several reports have noted that lindane also affects females reproductive function (Raizada et al., 1980; Uphouse 1987 and Cooper et al., 1989). Acute intoxication in vertebrates with the insecticide lindane has been found to produce an exaggerated responsiveness to sensory stimuli, hypersalivation, fasciculation of voluntary muscles, convulsions and death as a result of its neurotoxic effects (Joy, 1982). These effects most probably reflect a peripheral site of action of lindane, although there may also be a central increase in parasympathetic tone. It has also been suggested that the neurotoxic effect of lindane is due to its capacity to induce a hyperactivity of the central and peripheral cholinergic system, causing an increase in acetylcholine release from the cholinergic synaptic endings (Shankland, 1978). Moreover, variation in cerebral levels of tryptophan (Aldegunde et al., 1980), and other amino acid neurotransmitter such as glutamate, aspartate and or after γ-aminobutyrate (GABA) in response to lindane intoxication have also been reported (Munoz-Blanco et al., 1982 and 1984 and Cordoba et al., 1987).

Chickens are exposed to pesticides by different ways. Feeds are composed of several vegetable products that suffer treatment by pesticides to control parasites. This control may be made directly on the crop before or after the harvest, when feeds are transported or when they are stored in granaries. Another cause for the contamination, the direct treatment on the chickens by external application of dusts or sprays, the oral administration in the feed and the use of pesticides in chickens houses (Foster, 1974 and Lino and Noronha da Silvera, 1994).

Lindane is one of the few organochlorine pesticides left on the market. It has been used to treat animals and buildings for ectoparasites. It may enter the environment from industrial discharges, insecticide application or spills. Lindane is known to cause toxicity in animals but the toxicological effects of long exposure of lindane on chicken are still very inadequate. This work aimed to study the toxic effects of prolonged exposure to lindane insecticide in male and female chickens.

MATERIAL and METHODS

A total of thirty, three months old Balady Behary male and female chickens were maintained at the experimental houses using standard management practices. Chickens were acclimatized for 1 week before the beginning of the experiment. Feed and water were provided *ad libitum* to all birds throughout the experiment period.

Chemicals:

The gamma isomer of hexachlorocyclohexane (lindane, 99.5% purity) was obtained from the Merck-schuchardt Company, Hohenbrunn, Munich, Germany. Test kits used for different biochemical determinations were obtained from Boehringer Mannheim, Germany and Biomerieux, France.

Birds and experimental designs:

Chickens were divided randomly into three group (n=10, 5 males and 5 females). The first group was exposed with 5 ppm and the second with 10 ppm lindane in feed for 18 weeks. The third group was kept as control without any exposure. Body weight of each chicken as well as feed consumption were recorded before exposure and at weekly intervals during the investigation. Egg production and egg weights were recorded daily. After 18 weeks from exposure, all chickens were sacrificed by decapitation and free flowing blood was collected in clean vials for analysis. Liver, kidney, thyroid, thymus, spleen, hearts, proventriculus, gizzard, intestine, ovary and oviduct, testes, brain were dissected out and a postmortem morphological examination was performed. These organs were weighed as well as organ/body weight ratios were calculated. Serum was collected from the blood within two hours and analyzed for total protein, albumin, creatinine, acetylcholinesterase (AChE), aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and calcium and phosphorous for both lindane exposed birds as well as control non treated birds. Acetylcholinesterase enzyme was estimated in both serum and brain tissues.

Serum total protein, albumin, creatinine, AST, ALT activities and calcium and phosphorous were evaluated spectrophotometrically by using a Spectronic 1201 G 1001 plus Model 335489 with accessory printer model, 335488 Milton Roy Company, Spectrophotometer. Serum total protein (Weich Selbaum, 1949), albumin (Keay and Doxley, 1983), creatinine (Berndt, 1976)), enzyme analyses, (Reitman and Frankel, 1957) calcium (Gindler and King, 1972) and phosphorous (Morinal and Prox, 1973) were estimated at 30 °C, using commercially available diagnostic kits (Biommerieux Sa 45.903 000 F/Rcs, Toile/France). Serum globulin was determined by subtracting the values of albumin from the values of total proteins. Albumin/globulin ratio was determined by dividing the serum albumin value by the serum globulin values. Serum and brain acetylcholinesterase enzyme activity was determined spectrophotometrically using the method of Ellman et al. (1961).

Statistical analysis:

The obtained results were analyzed for the level of significance of the difference between control and exposed birds. This was assessed by employing Student's *t*-test and a *P* values of 0.05 or less was considered to be significant.

RESULTS

As shown in Tables 1 and 2 lindane given to chickens had a significant adverse effects on body weight gain, feed intake, egg production as well as egg weight compared with birds given normal feed. This effect was prominent in the birds of the group exposed to 10-ppm lindane.

Effect of lindane on relative organs weight was significant on thyroid, thymus, spleen, heart, ovary, oviduct, liver and gizzard of female and male birds especially of the 10 ppm treated group in comparison with the non-treated control group (Table 3).

As shown in Table 4, our results revealed a significant changes in total protein, albumin, globulin and creatinine concentrations in the serum of treated birds. A highly significant decrease in calcium and phosphorous concentration in the serum of lindane exposed chickens as compared with control were also recorded (Table 5). Pictures no. 1 and 2 show the effect of this alteration of calcium concentration on the egg shell.

Our results revealed a highly significant increase in AST in both male and female exposed chickens and ALT of male birds (Table 6). Our results presented in table 6 showed also a significant increase in brain acetylcholinesterase and highly significant increase in serum acetylcholinesterase activity of the exposed groups in comparison with the control non-treated group of birds.

DISCUSSION

Gamma hexachlorocyclohexane (γ-HCH) or lindane is a major organochlorine insecticide still used extensively in third-world countries for agriculture and for public health programs (Dikshith et al., 1991). Birds exposed to lindane in our expermint showed no obvious signs of toxicity alongwith the time of the investigation.

Lindane given to chickens had a significant adverse effects on body weight gain, feed intake, egg production and egg weight compared with birds given normal feed especially that group of birds treated with 10 ppm lindane (Tables 1 and 2). Our results revealed that the body weight gain in treated hens is significantly affected especially by the administration of 10 ppm of lindane in the ration, which reflects that the adverse effect of lindane on body weight was dose dependent. According to Rittich et al. (1983), lindane given to broilers at 1 or 10 mg/kg mixed feed had a significant adverse effect on weight gain, feed intake and feed conversion efficiency compared with birds not given lindane. According to Adamec et al. (1975), young chickens were exposed to gamma-BHC (lindane) at 2.5 to 25.0 mg/kg in the feed leading to decrease in the mass of the oviduct and serum calcium in comparison to control group of birds. These changes were attributed to the changes in the effectiveness of estrogen. The weight of the oviduct of birds exposed to insecticide was less by 12.7 to 17.1% than that of the control group and average concentration of serum calcium decreased by 8 to 27%. The authors concluded that the exposure of poultry to relatively low doses of chlorinated insecticides interferes with the biological effectiveness of sex hormones. A significant changes in relative weight of thyroid, thymus, spleen, heart, ovary, oviduct, liver and gizzard of female and male birds especially of the 10 ppm treated group in comparison with the non-treated control group (Table 3).

Our results revealed a significant changes in total protein, albumin, globulin and creatinine concentrations in the serum of exposed birds as compared with control (Table 4). These biochemical changes observed on the exposed birds suggest overt toxicity due to lindane treatment although there were no obvious clinical symptoms of toxicity have been recorded. In chicken given a ration containing 15 ppm lindane for 8 weeks starting at 3 weeks of age, total protein increased to the fourth week; after two and eight weeks the contents of amino-nitrogen were lower in experimental than in control birds (Manciulea et al., 1977).

The highly significant increase in AST in both male and female treated chickens and ALT of male birds (Table 6), is indicative of hepatotoxic effect of lindane and reflects the damage of hepatic tissue leading to leakage of soluble tissue enzymes as AST and ALT into the blood. These findings contradict that of Varshneya et al. (1986) who stated that the values of biochemical parameters in the serum of cockerels fed lindane with their diet were not altered, but they added that the livers from insecticide-treated birds showed varying degrees of degenerative changes and necrosis and there was hydropic degeneration, fatty degeneration and cytoplasmic vacuolization in hepatocytes. Our results support the hypothesis of alteration of the liver function of animals as well as birds exposed to chemicals (Dikshith et al., 1991). Weight of the liver after 8 weeks treatment was 15% lower than in control and liver glycogen content decreased by up to 71% after 6-8 weeks, while the activities of GPT and GOT increased 71 and 46% respectively but then decreased after 6 weeks (Giurgea et al., 1976). Technical hexachlorocyclohexane feeding to Swiss albino male mice at 500 ppm in diet for 100 days and 400 days respectively resulted in increased weight of heart, GOT, glucose and hypertrophic degeneration of aorta and myocardium (Shouche and Rathore, 1998).

An important part of the assessment of the safety of most chemicals is the chronic or long-term study, damage to gonads and their function can result from inhibition of overall hormonal controlling mechanisms at either the gonadal or the hypothalamic-pituitary level (Dixon and Hall, 1986). Lindane may act by inhibiting ATPase and thus limits the amount of energy available for the active transport of calcium into the shell gland. Highly significant decrease in calcium and phosphorous concentration in the serum of lindane treated chickens as compared with control were recorded in Table 5. Pictures no. 1 and 2 show the effect of this alteration of calcium concentration on the eggshell. These results reflect the effect of lindane on calcium metabolism of the treated birds. The thinning of eggshell could be explained as a disturbance in calcium metabolism either by disturbing the amount of calcium deposits controlled by the level of estrogen of the bird or due to failure in delivery of calcium to the shell controlled by carbonic anhydrase enzyme (Peakall, 1970). This thinning of the eggshell leads to destruction of these eggs as well as lowering their hatchability. These results lead to an explanation that lindane could change the balance of sex hormones in animals as well as in birds leading to reproductive failure in general and alteration of the calcium balance in the eggs in particular. In their study, Giurgea et al. (1976), gave chickens 3 weeks old 15 mg lindane/kg body weight each day for eight weeks and found that blood calcium content after 4 weeks was 16% and after 6 weeks 12% lower than in controls, returning to its normal after 8 weeks. Blood phosphorous content decreased about 14% after two weeks. Kosutzky et al. (1976) studied also the effect of lindane on sexual maturity, egg production and shell quality in chickens treated with HCH. Bobakova et al. (1976) proved some changes of biological activity of estrogen after exposure of chickens to some environmental pollutants including HCH and polychlorinated biphenyls (PCBs).

Our results presented in table 6 showed the effect of lindane on both serum and brain acetylcholinesterase activity in the given concentrations. From these results we can see that the administration of lindane in 5 and 10 ppm to chickens lead to a significant increase in brain acetylcholinesterase and highly significant increase in serum acetylcholinesterase activity in comparison with the control non exposed group of birds. This increase in acetylcholinesterase activity in brain is probably due to the direct or indirect activation of the central or peripheral cholinergic neurotransmitter system which, in turn, triggered off tonic-clonic convulsions in the acutely toxicated animals or birds. Furthermore, an increase in acetylcholinesterase activity correlated to an increase on acetylcholine turnover in CNS has long been sufficiently demonstrated (Cheney and Costa, 1977). The increase in acetylcholinesterase activity in both serum and CNS in our results suggests the involvement of cholinergic systems in lindane poisoning mechanism. Uchida et al. (1975) found that lindane toxicity increased acetylcholinesterase release on the cockroach abdominal ganglion. In this respect, our results suggest that the determination of acetylcholinesterase activity in serum may be used as a biochemical test to differentiate diagnosis between intoxication by organophosphate compounds or by lindane.

In spite of that the extrapolation of data from animals or birds to humans is inexact, we must warning about the toxic effects on human consumer's liver, metabolism, acetylcholinesterase activity as well as reproductive system almost all require further studies.

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Table 1: Effect of lindane on body weight gain percent (2 weeks on female and male) and feed intake(g)/hen/ day (mean ± SE).

Group No.	Dose	Time/2 weeks Sex	1 st	2 nd	3 rd	4 th	5 th	6th	7th	8th	9th
First	5 ppm	Female	10.6± 1.8	17.2 2.8	13.2± 1.9	10.5± 3.3*	10.2± 1.8	7.9± 0.9	1.2± 0.3***	0.2± 0.05***	2.3± 0.32**
Second	10 ppm		8.22± 2.6	11.2± 2.1**	5.1± 0.9***	10.1± 1.2*	10.4± 2.3*	6.1± 1.01*	1.0± 0.39***	0.5± 0.01***	-0.5± 0.1***
Third	Control		9.3± 2.55	16.7± 2.1	10.4± 1.8	12.6± 2.2	11.7± 2.1	8.6± 1.8	10.2± 1.9	4.1± 0.14	3.5± 0.13
First	5 ppm	Male	14.4± 1.7	19.7± 2.3*	15.1± 2.5**	16.7± 1.9**	13.6± 1.8	13.7± 1.5	15.8± 2.0*	8.7± 2.26**	7.8± 2.33**
Second	10 ppm		13.1± 2.2	16.5± 1.9***	13.1± 1.5***	13.4± 2.6***	10.3± 2.3*	7.9± 0.5**	9.1± 0.9***	4.9± 0.5***	-0.5± 0.1***
Third	Control		14.3± 2.3	22.3± 3.1	17.6± 2.5	18.7± 1.5	12.5± 1.7	13.7± 1.9	17.4± 2.2	15.3± 1.8	10.0± 2.1
First	5 ppm	Feed intake (both)	94.9 ±6.1***	102.3 ±0.9***	105.3 ±1.3***	116.8 ±0.8***	126.8 ±8.6	173.6 ±24.6	173.6 ±25***	128.9 ±7.0***	135.9 ±2.1***
Second	10 ppm		98.05 ±3.9***	102.7 ±0.8***	108.8 ±2.7***	112.8 ±4.4***	111.5 ±5.3***	191.4 ±37.1	177.5 ±46.9*	115.1 ±2.8***	120.5 ±0.7***
Third	Control		125.95 ±5.76	126.3 ±5.9	126.2 ±2.69	118.9 ±0.74	137.5 ±13.9	196.4 ±27.8	212.2 ±16.7	161.7 ±19.1	158.2 ±0.7

* Significant at P< 0.05
** Significant at P< 0.01
*** Significant at P< 0.001

Table 2: Effect of lindane on relative organs weight on female and male chickens (n=5 per group, mean ± SE).

Group No.	Dose	Sex	Thyroid	Thymus	Spleen	Kidney	Heart	Brain	R. Testes	L. Testes	Liver	Provent.	Gizzard	Intestine
First	5 ppm	Female	0.02± 0.0007**	0.13± 0.02***	0.15± 0.01*	0.44± 0.03	0.55± 0.02*	0.24± 0.0008	1.6± 0.36**	1.8± 0.5*	3.05± 0.2*	0.46± 0.05	2.4± 0.15*	5.65± 0.35
Second	10 ppm		0.01± 0.0003**	0.13± 0.01***	0.27± 0.08*	0.47± 0.03	0.51± 0.02***	0.25± 0.01	1.6± 0.29**	2.96± 0.68***	3.4± 0.27	0.59± 0.07*	2.8± 0.1	5.2± 0.28
Third	Control		0.03± 0.0	0.21± 0.04	0.23± 0.05	0.45± 0.03	0.58± 0.02	0.25± 0.0008	0.57± 0.3	1.25± 0.04	3.4± 0.2	0.48± 0.03	3.3± 0.8	5.6± 1.09
First	5 ppm	Male	0.01± 0.0002	0.08± 0.01	0.13± 0.0005	0.23± 0.02	0.63± 0.03*	0.16± 0.01	0.72± 0.3	0.66± 0.06	1.74± 0.1***	0.24± 0.02	2.16± 0.18*	2.5± 0.19
Second	10 ppm		0.01± 0.0	0.1± 0.02*	0.31± 0.13**	0.24± 0.01	0.51± 0.02***	0.22± 0.001**	0.73± 0.05	0.62± 0.03	1.99± 0.1***	0.27± 0.04	2.3± 0.06***	2.88± 0.5
Third	Control		0.01± 0.0	0.07± 0.01	0.12± 0.01	0.24± 0.01	0.58± 0.02	0.19± 0.01	0.76± 0.1	0.61± 0.1	1.36± 0.09	0.23± 0.0007	1.81± 0.17	2.38± 0.09

* Significant at $P < 0.05$
** Significant at $P < 0.01$
*** Significant at $P < 0.001$

Table 5: Effect of lindane on calcium (mg/dl), phosphorous (mg/dl), and calcium/phosphorous ratio (mean ± SE).

Group No.	Treatment	Ca (mg/dl)		P (mg/dl)		Ca/P ratio	
		Male	Female	Male	Female	Male	Female
First	5 ppm	7.28 ±0.22**	11.12 ±1.11***	6.48 ±0.71*	9.15 ±0.89**	1.17 ±0.11	1.23 ±0.10
Second	10 ppm	8.01 ±0.81*	11.23 ±0.56***	8.89 ±0.75	7.27 ±0.52***	1.27 ±0.16	1.50 ±0.06
Third	Control	10.10 ±1.29	15.15 ±1.04	8.22 ±0.99	11.03 ±1.06	1.28 ±0.14	1.39 ±0.09

Table 6: Effect of lindane on serum AST, ALT and serum and brain acetylcholinesterase enzymes (mean ± SE).

Group No.	Treatment	AST (IU/L)		ALT (IU/L)		Serum AChE		Brain AChE	
		Male	Female	Male	Female	Male	Female	Male	Female
First	5 ppm	216.75 ±8.56**	209.56 ±9.54**	28.18 ±0.79**	29.49 ±2.62	236.20 ±19.72***	237.54 ±26.24***	1229.75 ±55.04**	949.36 ±96.28*
Second	10 ppm	195.48 ±8.10*	188.44 ±12.20*	26.00 ±2.60***	30.36 ±2.63	210.30 ±23.17***	243.88 ±25.11***	818.98 ±108.82*	1082.15 ±81.79**
Third	Control	179.18 ±14.10	178.90 ±18.80	33.76 ±1.78	31.47 ±3.30	140.23 ±14.08	140.57 ±16.05	798.73 ±62.39	848.00 ±87.08

* Significant at $P< 0.05$
** Significant at $P< 0.01$
*** Significant at $P< 0.001$

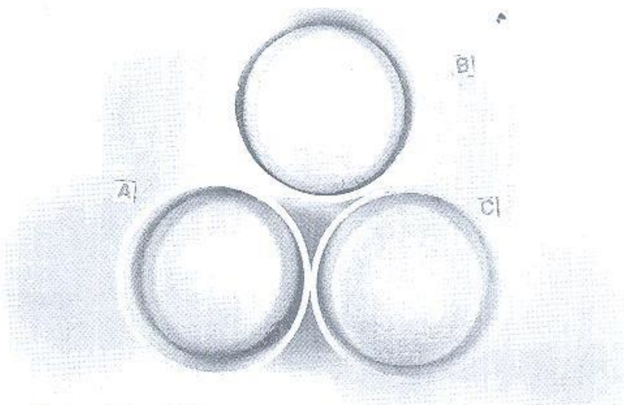
Table 3: Effect of lindane on egg weight (g) and egg production/hen/2 weeks (mean ± SE).

Group No.	Dose	Time/2 weeks Sex	1 st	2 nd	3 rd	4 th	5 th	6th	7th	8th	9th
First	5 ppm	Egg weight	-	-	-	26.30 ±0.4**	29.03±0.28**	30.37±0.8***	29.89±0.4***	39.59±0.53	37.49±0.9***
Second	10 ppm		-	-	-	28.24 ±0.8**	26.91±0.44**	29.07±0.5***	29.94±0.6***	39.14±0.41	39.40±0.53*
Third	Control		-	-	-	29.52 ±0.16	30.57±0.96	32.08±0.38	33.91±1.7	39.87±0.97	40.58±1.69
First	5 ppm	Egg production	-	-	-	2.13± 0.3***	2.00 ±0.4***	3.9 ±1.5***	8.73 ±1.42*	10.7 ±1.2*	10.9 ±0.7**
Second	10 ppm		-	-	-	4.13 ±1.98	3.25 ±0.4***	3.00 ±1.1***	4.12 ±0.8***	12.48 ±0.66	11.78 ±1.71
Third	Control		-	-	-	4.73 ±0.73	11.67 ±1.66	8.5 ±1.77	11.25 ±1.43	13.00 ±1.10	13.2 ±0.95

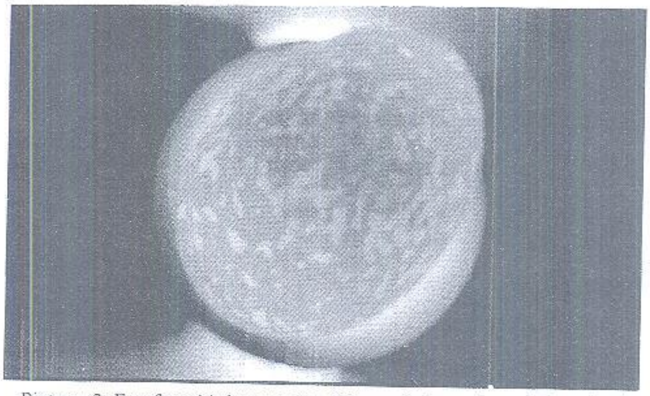
Table 4: Effect of lindane on total protein (g/L), albumin (g/L), globulin (g/L), and albumin/globulin ratio (mean ± SE).

Group No.	Dose	Total protein (g/L)		Albumin (g/L)		Globulin (g/L)		Al/Gl ratio		Creatinine mg/dl	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
First	5 ppm	31.83 ±1.13	36.19 ±1.46**	16.69 ±0.58	18.45 ±1.08*	15.14 ±1.58*	17.74 ±1.30***	1.17 ±0.18	1.11 ±0.12	1.89 ±0.09	1.84 ±0.14
Second	10 ppm	35.75 ±2.42**	37.59 ±3.31*	16.77 ±1.70	18.24 ±1.26*	20.29 ±1.87**	19.35 ±2.57***	0.84 ±0.09	1.03 ±0.10	2.69 ±0.2	2.46 ±0.34
Third	Control	31.01 ±1.61	33.41 ±0.62	18.22 ±1.56	19.90 ±0.84	11.50 ±1.84	13.51 ±0.26	1.31 ±0.20	1.19 ±0.20	1.97 ±0.13	1.64 ±0.18

* Significant at P< 0.05
** Significant at P< 0.01
*** Significant at P< 0.001



Picture 1: Eggshell thinning after lindane exposure
A- Control egg (no changes) B- 5 ppm lindane (mild change) C- 10 ppm lindane (severe changes)



Picture 2: Egg from bird exposed to 10 ppm lindane showed alteration in calcium deposition in eggshell