Punjab Univ. J. Zool., Vol. 11, pp. 67-81, 1996

HISTOPATHOLOGICAL CHANGES INDUCED BY AN ORGANOCHLORINE INSECTICIDE, DDT ON THE LIVER OF ALBINO RAT*

SYED SHAHID ALI AND ABDUL RAUF SHAKOORI

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: DDT was administered to albino rats as three doses (100 mg, 20 mg and 10 mg/kg B.Wt./day) for 48 hours, 15 days and 18 months durations, respectively. Animals were dissected after the stipulated periods, liver samples were removed and processed for histological analyses. Hepatic cell hypertrophy was the most common structural change induced by all doses of DDT. During 48 hour treatment, nuclear condensation, hyperchromatic and irregular shaped nuclei were observed. Clear zones around the nuclei and disorganized hepatic cord structure were also prominent changes. In 15 day DDT feeding dilation of sinusoidal spaces, enlarged blood vessels and bile canaliculi hyperchromatic nuclei, with increase in kupffer cells were noticed. Alteration in hepatic cords were also prominent in 6, 12 and 15 day treatments. During 18 month DDT feeding study somewhat degenerated (necrotic) zones alongwith irregular shaped hyperchromatic nuclei were observed. Highly hypertrophied kupffer cells were also present. Fatty degeneration and increased thickness of the cell membranes were also noticed in 12 and 18 months DDT treatments. Among the morphometric studies the increase in hepatic cell size, with decrease in number of cells/microscopic field with occasional rise in nuclear and nucleolarize were important findings in all three DDT feeding studies.

Key words: Hepatic morphology, morphometric changes, liver histology.

INTRODUCTION

Insecticides, heavy metals and chemical effluents from the industries are significant sources of pollution in the present world (Radulescu *et al.*, 1990; Shahida and Solangi, 1990; Jabbar *et al.*, 1991; Winter and Street, 1992; Rani *et al.*, 1993; Sukhoparova *et al.*, 1994; Vuorinen *et al.*, 1994; Urdaneta *et al.*, 1995). Among these pollutants, organochlorine group of insecticides is more dangerous as far as the environmental damage and degradation is concerned. DDT is one of these important organochlorine compound, which has been extensively used for controlling the pests of agriculture and public health importance (Metcalf, 1973; Floodstrom *et al.*, 1990; Gecheva, 1991; Bhatnagar *et al.*, 1992; Douthwaite, 1992; Aboul Ela *et al.*, 1993; Mourya *et al.*, 1993; Galassi *et al.*, 1994; Dirksen *et al.*, 1995).

Large amount of residues of DDT have constantly been detected and reported from

---

*This work was conducted by the financial assistance of Pakistan Science Foundation under the research project No.P-PU/Bio (93).
0079-8045/96/0001-0067 $ 03.00/0

all facets of life after its use for few years. The major cause of development of these residues was the persistant and highly stable nature of organochlorine compounds including DDT (Mohammad *et al.*, 1990; Calero *et al.*, 1992; Rao, 1992; Singh *et al.*, 1992; Mason and Madsen, 1993; Thao *et al.*, 1993).

Number of xenobiotic compounds have the ability to induce various type of pathologies in animal tissues. Organochlorine compounds are of great significance among those (Roberts and Robert, 1982; Kandalaft *et al.*, 1991; Jonsson *et al.*, 1993; Keith *et al.*, 1993; Katayama, 1993; Teshke *et al.*, 1993; Srivastava and Srivastava, 1994). These compounds also produced hepatotoxic effect, which has also been reported from this and other laboratories (Shakoori *et al.*, 1982, 1984; Ali *et al.*, 1988; Gupta *et al.*, 1989; Ali and Shakoori, 1990, 1993; Bagchi *et al.*, 1993; Misutani *et al.*, 1994; Begum and Vijayara-ghavan, 1995).

Conflicting reports about the carcinogenic potential of DDT are also found in literature (Turusov *et al.*, 1973; Kashyap *et al.*, 1977; Rossi *et al.*, 1977; Flodstrom *et al.*, 1990; Yusof and Edwards, 1990; Adenuga *et al.*, 1992).

The objective of present report was to study the histopathological and morphometric effects of various sublethal doses of DDT on liver, administered for short and long term durations.

MATERIALS AND METHODS

Sprague Dawley rats reared in the Animal House of Department of Zoology were used for the experiment. About 5 month old animals were used for short term experiments, while younger rats about 3 months old were used for long term experiment.

Five to six animals were caged in 2.5 cubic feet iron cages and provided with food and water *ad libitum*. Laboratory prepared rat feed was used for the study, the composition of which has already been mentioned (see, Ali and Shakoori, 1990).

Administration of insecticide

An insecticide belonging to chlorinated hydrocarbon group, DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl ethane), collected as 75% powder from Agriculture Department, Government of the Punjab, was used for this study. The toxicant was administered to animals alongwith the feed as three different doses for variable durations *i.e.*, 100, 20 and 10 mg/kg body weight/day for 48 hour, 15 day and 18 month durations, respectively. In first short term (ST-I) experiment, 800 mg DDT (75%) was added per kg of dry feed. In second case (ST-II experiment) 525 mg of DDT was mixed with 3 kg of feed while in long term (LT) case, 87.5 mg of DDT was thoroughly mixed per one kg of dry rat feed, with small amount of water to prepare thick cakes of feed.

Procedure adopted

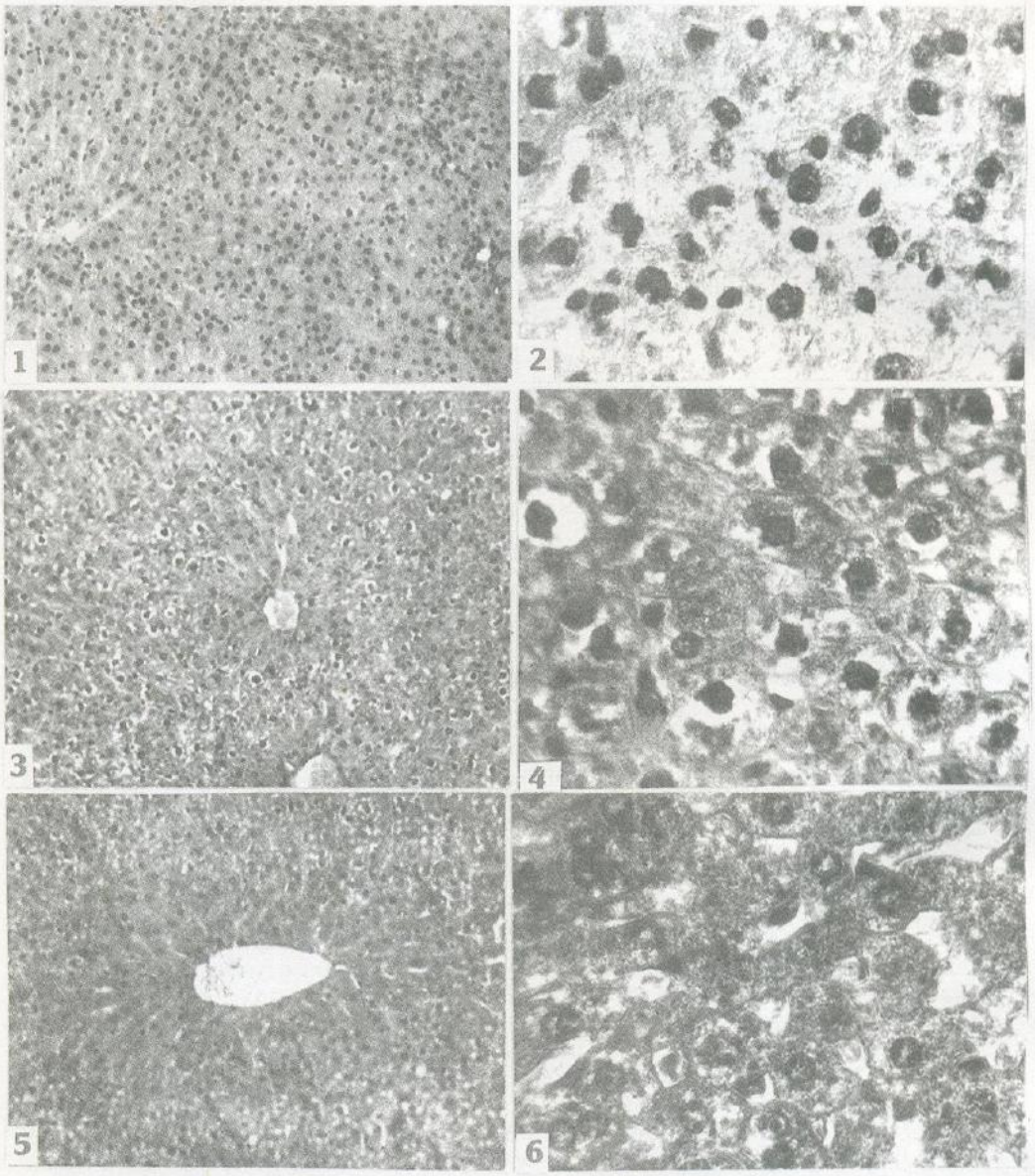
A strong dose of DDT (100 mg/kg B.Wt./day) was fed to a group of 4 rats for short term (48 hours) duration. Two weak doses (20 mg and 10 mg/kg/day) were administered to two other groups of rats for 15 days and 18 months, respectively. A control group was also treated similarly, except insecticide treatment for each experiment.

After the stipulated periods of 24 and 48 hours in strong dose experiment, after 3, 6, 9, 12 and 15 days in 1st week dose case and after 6, 12 and 18 months duration in 2nd week dose experiment, 3-6 animals were anaesthetized and dissected from both DDT treated and control groups. Their liver samples were quickly removed and fixed in Bouin's fixative.

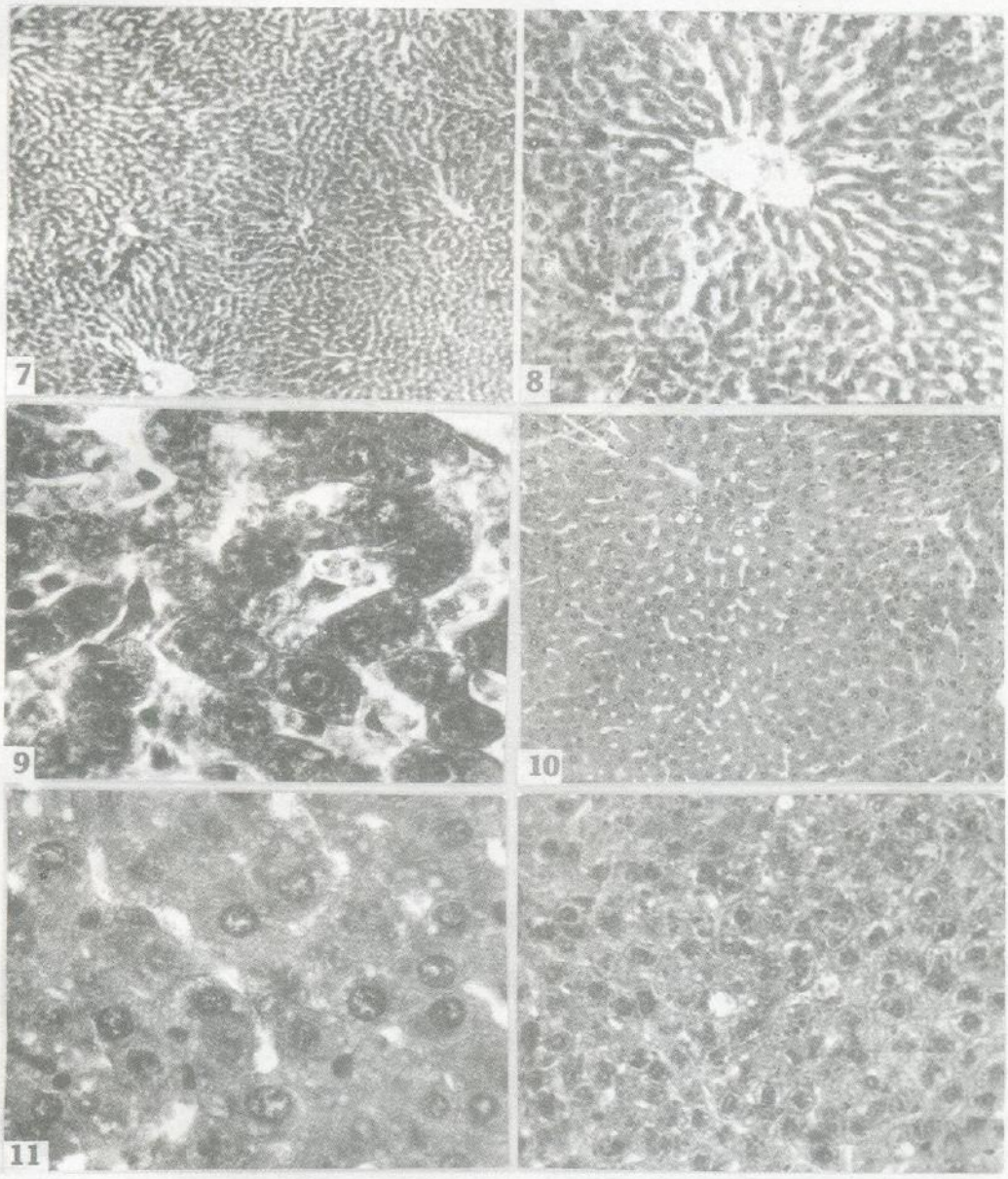
The liver samples were further processed for microtomy using routine histological techniques. About 6-8 μm thick liver sections were prepared and stained with haematoxyline and eosin differential staining technique, which were further studied for various histological changes. The liver sections were also studied for different morphometric parameters, which include the size of hepatic cell, nuclei and nucleoli, number of cells per microscopic field, number of nuclei per cell and number of nucleoli per nucleus. Ocular micrometer was used for measuring the size of cell and its components which was initially calibrated with stage micrometer.

RESULTS

Tables I-III and Figures 1-24 show the effect of various doses of DDT (100 mg, 20 mg and 10 mg/kg/day) on the morphometric and histological structure of liver. The hepatic cells increased in size in both short-term treatments. This increase was 29% in 24 hours feeding group and 23% in 48 hour feeding case. In second ST experiment this increase was 14-26%. In 18 months (long-term) DDT treatment similar change in hepatic cells was also found with 27-45% increase. The nucleolar size in this experiment increased significantly which was 56% in 24 hour and 44% in 48 hour feeding group. The number of nuclei/cell and number of nucleoli/nucleus remained unaffected in all three treatments. The number of cells/field showed significant decrease during both ST experiments. This decrease was 16 and 22% during 48 hours and 17-28% in 15 day treatments. The size of the nucleus remained unchanged in first ST experiment while in second ST experiment when it was increased by 18% and showed 13% rise in 15 day treatment. The nucleolar size did not show any significant change except 29% increase at day 3 in 15 day DDT feeding experiment. Table III shows the effect of long term feeding of DDT on the various histological parameters of rat liver. As is typical of all the chlorinated insecticide toxicity in non-target organisms, the hepatic cells showed hypertrophy. The hepatic cell size increased 33%, 27% and 45% with simultaneous decline in number of cells/microscopic field with 22, 21 and 29% decrease after 6, 12 and 18 months of feeding, respectively. The nuclear size of hepatic cells increased significantly (20%) after 18 months of insecticide feeding. The nucleolar size, on the other hand, remained unaltered.



Figs.1-6. Histological structure of normal rat liver showing hepatic sinusoidal spaces (Fig.1), normal hepatic morphology (Figs.1-2). The hepatic structure of DDT-fed animals for 24 hour (Figs.3-4) and 48 hours (Figs.5-6) are also shown. Note disturbed sinusoidal areas and hepatic cords (Fig.3), clear areas in the cytoplasm around nuclei and hypertrophy of hepatocytes (Figs.3-4), darkly stained oval area around central vein, and fine clear areas in the tissues (Fig.5), hypertrophied cells, nuclei with large clear areas around nucleus and in cytoplasm (Fig.6). Magnification: Figs. 1, 3, 5, 50X; Figs. 2, 4, 6, 250X. Stain: haematoxylin and eosin.



Figs.7-12. Histological structure of rat liver fed on DDT-mixed diet for 3 days (Figs.7-9), 6 days (Figs.10-11) and 9 days (Fig.12). Note altered lobular morphology (Figs. 7-10), increase in number of kupffer cells (Figs. 8-9), enlarged sinusoidal areas (Figs. 8, 9, 11), cellular and nuclear hypertrophy (Figs.9-10) and slight vacuolation (Figs.10-12). Magnification: Fig.7, 25X; Figs. 8, 10, 50X; Fig. 12, 100X; Figs. 9, 11, 250X; Stain: haematoxylin and eosin.

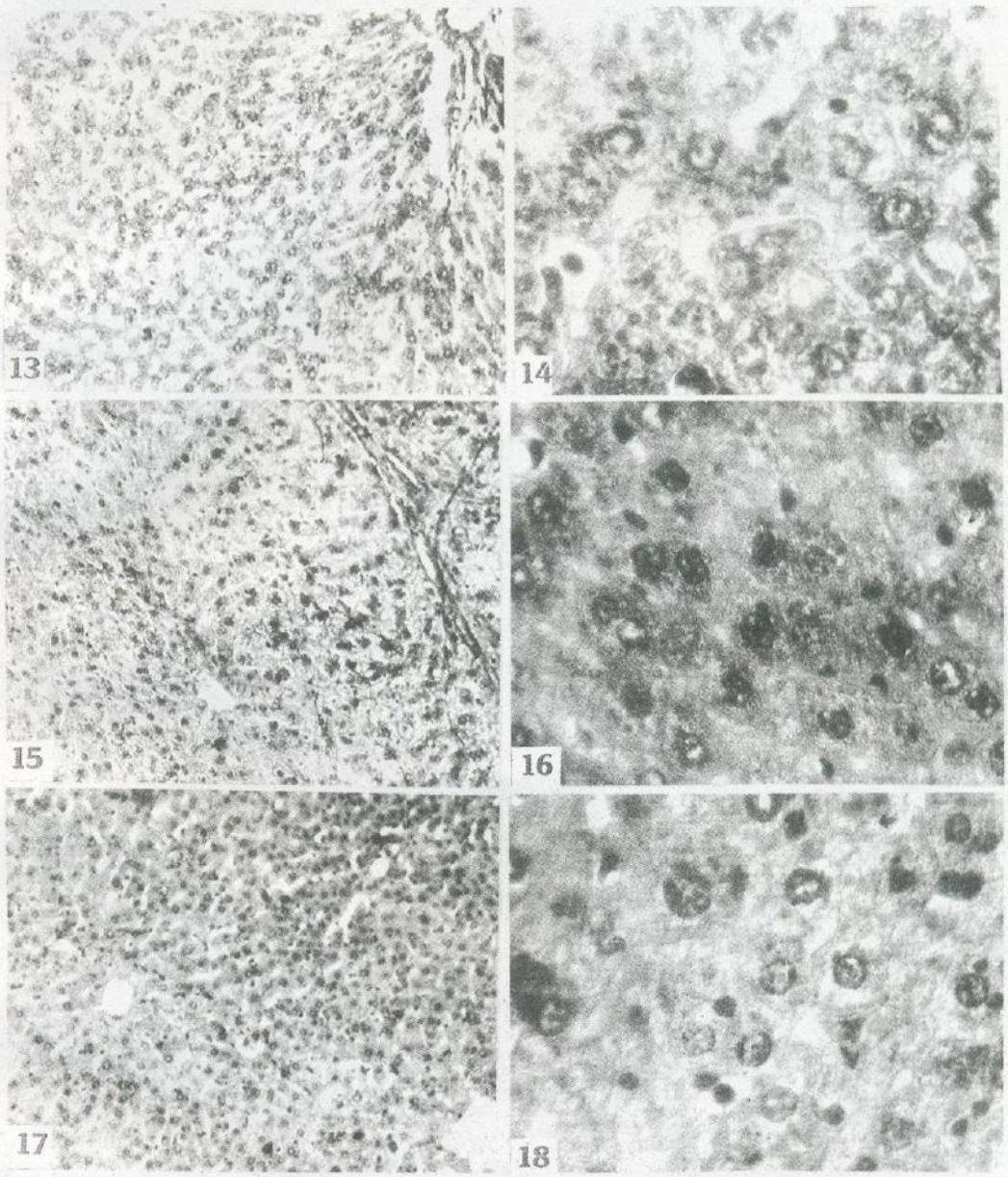
Figures 3 to 5 showed histological structure of liver of rat fed on DDT mixed diet for 24 hours, while Figures 5 and 6 showed histological changes after 48 hours of DDT feeding when compared with control liver (Figs.1-2). The hepatic cells and nucleoli showed hypertrophy. The nuclei in 24 hour group were condensed with a clear space around them. The blood vessels and bile canaliculi were prominently enlarged (compare Figure 3 with Figure 1). In 48 hour treatment the nuclei were distinctly prominent and vesicular with irregular clear zones in the tissue (Fig.6). The general hepatolobular architecture showed abnormalities and necrotic region on the periphery (Figs.3-6), when compared with control liver structure (Figs.1-2).

Prominent changes in hepatic structure of rat were observed following DDT feeding for 3 days (Figs.7-9) 6 days (Figs.10-11), 9 days (Fig.12), 12 days (Figs.13-14) and 15 days (Figs.15-16). The hypertrophied cells, well defined vesicular nuclei enlarged kupffer cells and sinusoidal areas (Figs.9, 11, 14, 16, 18) swollen bile canaliculi and other blood vessels (Figs.7-8, 17) when compared with control (Figs.1-2) were the prominent structural changes in liver with increasing duration of DDT administration. The cellular hypertrophy and formation of vesicular nucleus (Figs.11, 16, 18) became more prominent and disorganized lobular zones with disruption of hepatic cord structure (Figs. 10 and 12-15) and fatty degeneration (Figs.11-13) were further increased with increase in duration of treatment.

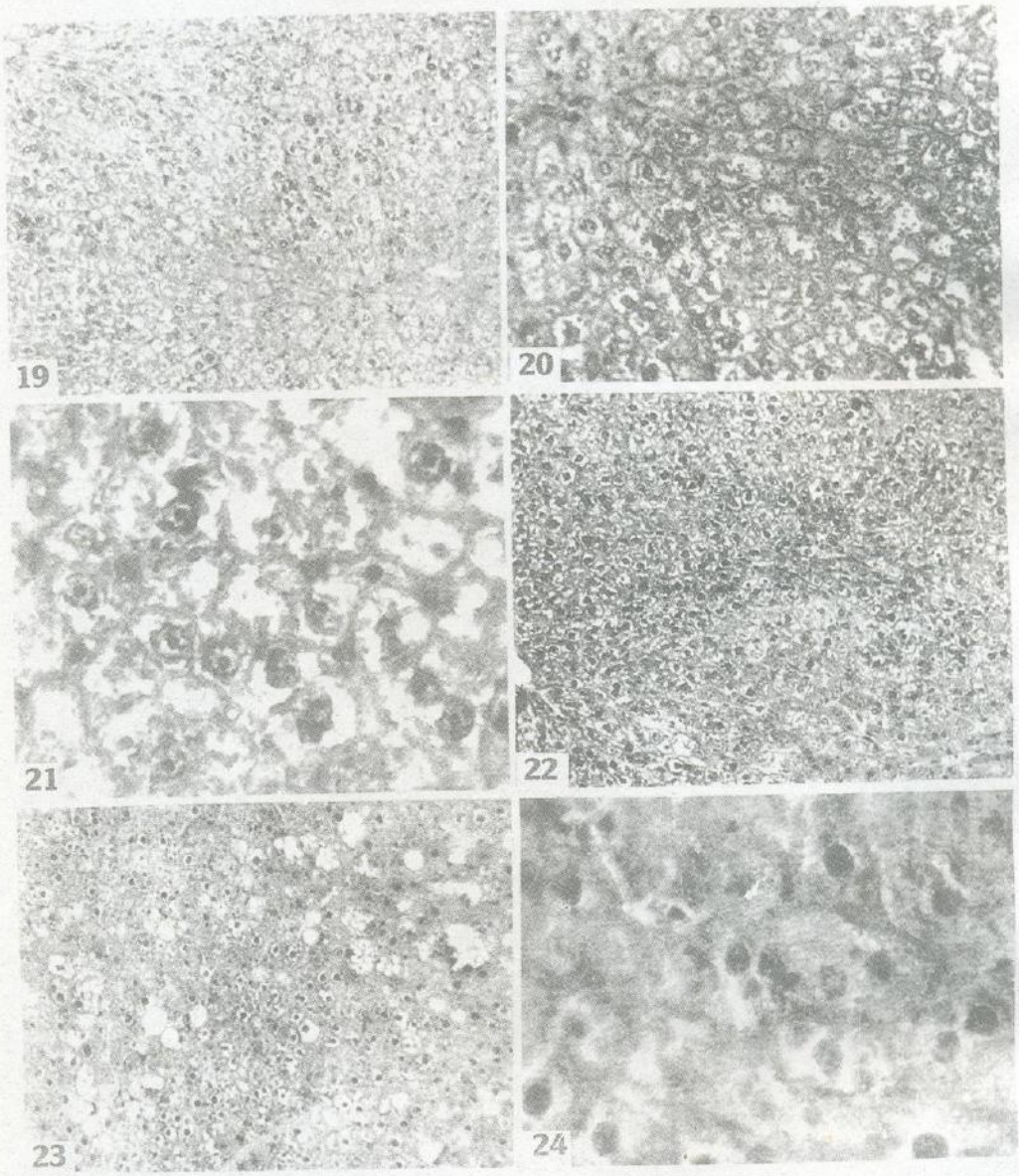
Table 1: Effect of feeding DDT-mixed diet (100 mg/kg body weight/day) for 48 hours on various histological parameters of rat liver.

Parameters	Control	DDT-fed	
		24 hours	48 hours
No. of cells/ field (n=9)	291.42[a] ±16.73	244.55* ±9.78	228.49** ±13.52
No. of nuclei/ cell (n=90)	1.10 ±0.08	1.15 ±0.05	1.18 ±0.07
No. of nucleoli/ nucleus (n=90)	1.58 ±0.16	1.78 ±0.25	1.72 ±0.16
Size of cell (μ^2; n=90)	281.77 ±11.46	363.72*** ±9.02	345.57*** ±6.30
Size of nucleus (μ^2; n=90)	39.57 ±2.04	37.54 ±1.18	40.98 ±1.34
Size of nucleolus (μ^2; n=90)	2.52 ±0.34	3.93** ±0.38	3.62* ±0.26

Mean ± SEM. Student's 't' test; *: P<0.05; **: P<0.01; ***: P<0.001.



Figs.13-18. Histological structure of rat liver fed on DDT-mixed diet for 12 days (Figs.13-14), 15 days (Figs.15-16) and 6 months (Figs.17-18). Note highly disorganised cord morphology (Figs. 13, 15, 17) fairly abnormal sinusoidal areas with numerous round and swollen kupffer cells (Figs. 13, 16, 17, 18), hypertrophied (Figs. 14, 18), irregular shaped condensed nuclei (Figs. 15-17) and distorted blood vessels (Figs. 15, 17). Magnifications: Figs. 13, 15, 17, 50X; Figs. 14, 16, 18, 250X; Stain: haematoxylin and eosin.



Figs.19-24. Histological structure of rat liver fed on DDT-mixed diet for 12 months (Figs.19-21) and 18 months (Figs.22-24). Note disturbed lobular and cord morphology distinctly prominent membrane network (Figs. 19-20), cytoplasmic margination (19-23), extensive vacuolation (Fig.23), hypertrophied (Fig.21) and irregular shaped condensed nuclei (Figs. 20, 21, 24). Magnification: Figs. 19, 22, 23, 50X; Fig. 20, 100X and Figs. 21, 24, 250X. Stain: haematoxylin and eosin.

Table II: Effect of feeding DDT mixed diet (20 mg/kg body weight/day) for 15 days on the various histological parameters of albino rat liver.

Parameters	Control	DDT-fed				
		3 days	6 days	9 days	12 days	15 days
No. of cells/ field (n=9)	267.44[a] ±15.13	242.39 ±11.19	221.29* ±9.48	232.61 ±12.77	205.61** ±10.37	193.48*** ±9.87
No. of nuclei/ cell (n=90)	1.12 ±0.04	1.25 ±0.06	1.15 ±0.07	1.12 ±0.04	1.19 ±0.04	1.23 ±0.09
No. of nucleoli/ nucleus (n=90)	1.58 ±0.15	1.60 ±0.14	1.82 ±0.13	1.80 ±0.24	1.70 ±0.11	1.80 ±0.25
Size of cell (μ^2; n=90)	270.63 ±11.21	298.36 ±8.05	308.18** ±7.80	309.12** ±6.25	334.82*** ±8.05	341.05*** ±15.13
Size of nucleus (μ^2; n=90)	43.34 ±1.20	45.40 ±1.75	44.36 ±1.47	46.98 ±1.56	51.09*** ±1.55	48.87** ±1.35
Size of nucleolus (μ^2; n=90)	2.87 ±0.21	3.70* ±0.25	3.35 ±0.23	3.26 ±0.36	3.80 ±0.45	3.09 ±0.60

[a]Mean ± SEM. Student's 't' test; *: P<0.05; **: P<0.01; ***: P<0.001.

On comparison of Figures 17-18 (6 month feeding), 19-21 (12 months feeding) and 22-24 (18 months feeding) with figures 1-2 (control group) several histological changes can be observed. Although the general hepatolobular architecture was maintained the hepatic cells showed hypertrophy (Figs.20, 21, 24) after 12 and 18 months of DDT feeding. The nuclei became well demarkated irregular shaped and condensed (Figs.17, 20 and 22). The plasma membranes of hepatic cells and bile canaliculi also became very prominent (Figs.20-21). Eighteen and twelve months of DDT feeding also resulted in appearance of numerous vacuoles in the hepatic tissue (Figs. 20, 21 and 23) which could be fatty degeneration of hepatic tissue and are indicative of its toxicity. The hepatic nuclei also became very much condensed and were surrounded by a clear zone (Figs. 19-23).

Table III: Effect of feeding DDT mixed diet (10 mg/kg body weight/day) for a total period of 18 months on the various histological parameters of rat liver.

Parameters	6 months		12 months		18 months	
	Control	DDT fed	Control	DDT fed	Control	DDT fed
No. of cells/ field (n=9)	238.37[a] ±11.68	184.78** ±10.49	269.41 ±12.40	213.90** ±14.41	244.64 ±10.71	174.81** ±13.81
No. of nuclei/ cell (n=90)	1.07 ±0.06	1.17 ±0.08	1.11 ±0.04	1.17 ±0.08	1.04 ±0.03	1.12 ±0.04
No. of nucleoli/ nucleus (n=90)	1.52 ±0.16	1.88 ±0.31	1.46 ±0.14	1.63 ±0.21	1.40 ±0.19	1.62 ±0.12
Size of cell (μ^2; n=90)	290.31 ±8.78	387.12*** ±15.76	258.61 ±14.17	328.34*** ±6.84	273.44 ±11.19	395.56*** ±11.37
Size of nucleus (μ^2; n=90)	37.91 ±1.23	40.96 ±1.50	41.72 ±1.81	45.92 ±1.57	43.69 ±1.94	52.26* ±2.88
Size of nucleolus (μ^2; n=90)	2.79 ±0.21	3.29 ±0.22	2.94 ±0.30	3.24 ±0.32	3.11 ±0.24	3.50 ±0.39

[a]Mean ± SEM. Student's 't' test: *: P<0.05; **: P<0.01; ***: P<0.001.

DISCUSSION

The DDT feeding results in hypertrophy of hepatic cell and increase in its nuclear and nucleolar size. In 48 hour feeding experiment the cell size showed about 23% increase, while nucleolus showed 44% increase after 48 hours of DDT feeding. In 15 day feeding experiment the cell size increased upto 26% while nucleus showed 18% increase. In 18 month feeding experiment, this increase was respectively, 45% and 20%. The number of cells per microscopic field decreased by 22%, 28% and 29% at 48 hours, 15 days and 18 months feeding experiment. The number of nuclei and nucleoli remained unaltered.

Besides these changes in size of hepatic cell and its components, typical hepatic damage, as manifested in the form of sinusoidal congestion, cellular vacuolation and foamy appearance, was evident in the present study. Similar type of morphological changes have been reported from other labortories (Datta and Dikshith, 1973). Ramalingam (1985) has found harmful effects of DDT on liver structure which may lead to alterations in tissue metabolism. DDT treatment produced moderate fatty

Table IV: **Effect of feeding DDT mixed diet for three different durations on the various histological parameters of rat liver. The values are shown as percent increase (+) or decrease (-) with reference to their respective controls.**

Parameters	DURATION OF DDT TREATMENT									
	Hours		Days					Months		
	24	48	3	6	9	12	15	6	12	18
Cell size	+29***	+23***	+10	+14**	+14**	+24***	+26***	+33***	+27***	+45***
Nuclear size	-5	+4	+5	+2	+8	+18***	+13**	+8	+10	+20*
Nucleolar size	+56**	+44*	+29*	+17	+14	+32	+8	+18	+10	+13
No. of cells/ field	-16*	-22**	-9	-17*	-13	-23**	-28***	-22**	-21**	-29**
No. of nuclei/ cell	+5	+7	+12	+3	-	+6	+10	+9	+5	+8
No. of nucleoli/ nucleus	+13	+9	+3	+17	+15	+9	+15	+24	+12	+16

* P<0.05; **: P<0.01; ***: P<0.001 (Student's 't' test).

degeneration, ultrastructural changes in the mitochondria, endoplasmic reticulum and lysosome in rat liver with maximum on day 60 (Kaloyanova-Simeonova *et al.*, 1983). It has been concluded in this study that after day 90, the adaptive process restricts the further damage. Kimbrough *et al.* (1971) have shown that 250 ppm DDT dose, given to rats, caused enlargement of the hepatic cells around the central veins and cytoplasm has smooth appearance. The dietary concentration of 500 ppm caused cytoplasmic inclusions in number of hepatic cells and the enlargement of the cells (hypertrophy) had spread to a larger portion of the liver lobules. Margination and moderate number of vacuolated cells were also observed at this dose. These findings can also be correlated with the present studies. Similar changes in hepatic structure following oral administration of other organochlorine insecticides has already been reported from this laboratory (Ali and Shakoori, 1990 1993). DDT also induced changes in other tissues of animals. Baronia and Sahal (1993) reported induction of necrosis in seminiferous tissue hypertrophy in germinal epithelium of sperm cells shrinkage of spermatogonia, spermatocytes and spermatids following DDT feeding (@ 500 mg/kg B.Wt.) for six weeks in albino rats. Although, DDT-induced carcinogenic changes in liver were not reported during this study. There are considerable evidences that DDT proved carcinogenic in different animals (Thorpe and Walker, 1973; Kashyap *et al.*, 1977), while Laws (1971) concluded that DDT has antitumorigenic properties in a controlled experimental tumour system.

REFERENCES

ABOUL ELA, R.G., MORSY, T.A., EL-GOZAMY, B.M.R. AND RAGNER, D.A., 1993. The susceptibility of the Egyptian *Phlebotomus papatasi* to five insecticides. *J. Egypt. Soc. Parasitol.*, **23**(1): 69-94.

ADENUGA, G.A., BABABUNMI, E.A. AND HENDRICK, R.G., 1992. Depression of the Ca^{++} ATPase activity of the rat liver endoplasmic reticulum by the liver tumor promotors 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane and phenabarbital. *Toxicology*, **71**(1-2): 1-6.

ALI, S.S. AND SHAKOORI, A.R., 1990. Toxicology of aldrin in rats. *Punjab Univ. J. Zool.*, **5**: 1-56.

ALI, S.S. AND SHAKOORI, A.R., 1993. Short-term toxicity of endrin in Sprague Dawley rats. Biochemical and histological changes in liver. *Punjab Univ. J. Zool.*, **8**: 1-13.

ALI, S.S., ALI, N.S. AND SHAKOORI, A.R., 1988. Biochemical alterations induced by short-term feeding of endrin on various blood components of albino rats. *Proc. Pakistan Congr. Zool.*, **8**: 101-112.

BAGCHI, M., HASSOUN, E.A., BAGCHI, D. AND STOHS, S.J., 1993. Production of reactive oxygen species by peritoneal macrophages and hepatic mitochondria and microsomes from endrin-treated rats. *Free Radical Biol. Med.*, **14**(2): 149-155.

BARONIA, A.K. AND SHAHI, Y.N., 1993. DDT induced changes in the testis of albino rat: A histopathological study. *J. Environ. Biol.*, **14**(2): 153-156.

BEGUM, G. AND VIJAYARAGHAVAN, S., 1995. *In vivo* toxicity of dimethoate on

protein and transaminases in the liver tissue of freshwater fish, *Clarias batrachus* (Linn.). *Bull. Environ. Contam. Toxicol.*, **54**(3): 370-375.

BHATNAGAR, V.K., PATEL, J.S., VARIYA, M.R., VENKAIAH, K., SHAH, M.P. AND KASHYAP, S.K., 1992. Levels of organochlorine insecticides in human blood from Ahmadabad (Rural), India. *Bull. Environ. Contam. Toxicol.*, **48**(2): 302-307.

CALERO, S., FOMSGAARD, I., LACAYO, M.L., MARTINEZ, V. AND RUGAMIA, R., 1992. Preliminary studies of 15 organochlorine pesticides in lake Xolothan, Nicaragua. *Chemosphere*, **29**: 1413-1419.

DATTA, K.K. AND DIKSHITH, T.S.S., 1973. Histopathologic changes in the testis and liver of rats repeatedly exposed to pesticides. *Exp. Pathol.* (Jena), **8**: 363-370.

DIRKSEN, S., BOUDEWIJN, T.J., SLAGER, L.K., MES, R.G., VAN SCHAICK, M.J.M. AND DEVOOGL, P., 1995. Reduced breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. *Environ. Pollut.*, **88**(2): 119-132.

DOUTHWAITE, R.J., 1992. Effects of DDT treatments applied for tsetse fly control on white-browed sparrow-weaver (*Plocepasser mahali*) populations in NW Zimbabwe. *Afr. J. Ecol.*, **30**(3): 233-244.

FLODSTROM, S., HEMMING, H., WEARNGAARD, L. AND AHLBORG, U.G., 1990. Promotion of altered hepatic foci development in rat liver, cytochrome P-450 enzyme induction and inhibition of cell-cell communication by DDT and some structurally related organohalogen pesticides. *Carcinogenesis*, **11**(8): 1413-1417.

GALASSI, S., GUZZELLA, L., BATTEGAZZORE, M. AND CARRIERI, A., 1994. Biomagnification of PCB's, p,p.DDE and HCB in the river PO Ecosystem (Northern Italy). *Ecotoxicol. Environ. Saf.*, **29**(2): 174-186.

GECHEVA, G., 1991. Resistance of German cockroach (*Blattela germinica*) to DDT and propoxur in some parts of Bulgaria. *Wiad. Parazytol.*, **37**(3): 367-373.

GUPTA, P.H., MEHTA, S. AND MEHTA, S.K., 1989. Effect of DDT on hepatic mixed-function oxidase activity in normal and vitamin-A-deficient rats. *Biochem. Int.*, **19**(2): 247-256.

JABBAR, A., AKHTAR, M.S. AND ALI, M., 1991. Soil and aquifer pollution by agrochemicals in Pakistan. *Progress. Farm.*, **11**: 40-44.

JONSSON, C.M. AND TOLEDO, M.C.F., 1993. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. *Arch. Environ. Contam. Toxicol.*, **24**(2): 151-155.

KALOYANOVA-SIMEONOVA, F., KRUSTEV, L. AND TASHEVA, M., 1983. Ultrastructural changes in albino rat liver after 3 month DDT treatment. *Probl. Khig.*, **8**: 50-56.

KANDALAFT, K., LIU, S., MANIVEL, C., BORNER, J.W., DRESSEL, T.D., SUTHERLAND, D.E.R. AND GOODALE, R.L., 1991. Organophosphate

increases the sensitivity of human exocrine pancreas to acetylcholine. *Pancreas*, **6**(4): 398-403.

KATAYAMA, T., 1993. Effect of dietary addition of myo-inositol on metabolic changes in rats exposed to 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane. *Nutrition Res.*, **13**(4): 445-454.

KEITH, J.O. AND MITCHELL, C.A., 1993. Effects of DDE and food stress on reproduction and body condition of ringed turtle doves. *Arch. Environ. Contam. Toxicol.*, **25**(2): 192-203.

KIMBROUGH, R.D., GAINES, T.B. AND LINDER, R.E., 1971. The ultrastructure of livers of rats fed DDT and dieldrin. *Arch. Environ. Hlth.*, **22**: 460-467.

LAWS, E.R. Jr., 1971. Evidence of anti-tumorigenic effects of DDT. *Arch. Environ. Hlth.*, **23**: 181-184.

MASON, C.F. AND MADSEN, A.B., 1993. Organochlorine pesticide residues and PCBs in Danish otters (*Lutra lutra*). *Sci. Total. Environ.*, **133**(1-2): 73-81.

METCALF, R.L., 1973. A century of DDT. *J. Agric. Food Chem.*, **21**: 511-519.

MIZUTANI, T., NAKAHORI, Y. AND YAMAMOTO, K., 1994. p-Dichlorobenzene-induced hepatotoxicity in mice depleted of glutathion by treatment with buthionine sulfoxime. *Toxicology*, **94**(1-3): 57-67.

MOHAMMAD, A., EKLUND, A., OESTLUND-LINDQUIST, A. AND SLANINA, P., 1990. Distribution of toxiphene, DDT and PCB among lipoprotein fractions in rat and human plasma. *Arch. Toxicol.*, **64**(7): 567-571.

MOURYA, D.T., GOKHALE, M.D., CHAKRABORTI, S., MAHADEV, P.V.M. AND BANERJEE, K., 1993. Insecticide susceptibility status of certain populations of *Aedes aegypti* mosquito from rural areas of Maharashtra state. *Indian J. Med. Res. Sect A* **97** (March): 87-91.

RADULESCU, V., RAUTA, C., PERE, N. AND CHIRIBELEA, C., 1990. The dynamics of organochlorine insecticide residues in soil and their plant uptake. *An. Inst. Cercet. Pedol. Agrochim.*, **50**: 255-270.

RAMALINGAM, K., 1985. Effects of DDT and melathion on tissue succenic dehydrogenase activity (SDH) and betic dehydrogenase isoenzymes (LDH) of *Sarotherodon mossambicus* (Peters). *Proc. Indian Acad. Sci. Anim. Sci.*, **94**: 527-531.

RANI, B., AMITA, E., KRISHNAKUMAR, M.K. AND KARANTH, N.G.K., 1993. Metabolism and embryotoxicity of heptachlor in the albino rat (*Rattus norvegicus*). *J. Environ. Biol.*, **14**: 77-87.

RAO, V.S., 1992. Organochlorine pesticide residues in a village drinking water pond (Pusulur) of Guntur (District) of Andhra Pradesh. *Geobios (Jodhpur)*, **19**(5): 200-203.

ROBERTS, M.H. Jr. AND ROBERT, E.B., 1982. Acute toxicity of Kepone to selected freshwater fishes. *Estuaries*, **5**(3): 158-164.

ROSSI, L., RAVERA, M., REPETTI, G. AND SANTI, L., 1977. Long term administration of DDT or phenoharbital-Na in Wistar rats. *Int. J. Cancer*, **19**: 179-185.

SHAHIDA, A. AND SOLANGI, A., 1990. Effects of pesticides on non-target soil micro-organisms: a review. *Progress. Farm.*, **10**: 25-29.

SHAKOORI, A.R., RASUL, Y.G. AND ALI, S.S., 1982. Effect of dieldrin feeding for 6 months on albino rats biochemical and histological changes in liver. *Pakistan J. Zool.*, **14**: 191-204.

SHAKOORI, A.R., RASUL, Y.G. AND ALI, S.S., 1984. The effect of long term administration of dieldrin on biochemical components of blood serum of albino rats. *Folia Biol (Krakow)*, **32**: 213-222.

SINGH, G., DOWMAN, A., HIGGINSON, F.R. AND FENTON, I.G., 1992. Translocation of aged cyclodiene insecticide residues from soil into forage crops and pastures at various growth stages under field conditions. *J. Environ. Sci. Health Part B Pestic Food Contam. Agric. Wastes*, **27**(6): 711-728.

SRIVASTAVA, A.K. AND SRIVASTAVA, A.K., 1994. Effects of chlordecone on the gonads of freshwater catfish. *Heteropneustes fossillis*. *Bull. Environ. Contam. Toxicol.*, **53**(2): 186-191.

SUKHOPAROVA, V.P., SOKOLOV, O.A., TYURYUKANOVA, STREKOZOV, B.P., PERIFILOVA, N.V. AND ZLOBINA, A.L., 1994. Organochlorine compounds and heavy metals in fish of the upper Oka basin. *Russ. J. Ecol.*, **25**(1): 30-35.

TESHKE, K., KELLY, S.J., WIENS, M., HERTZMAN, C., WARD, H.D., WARD, J.E.H. AND VAN-OOSTDAM, J.C., 1993. Concentrations of organochlorine pesticides in the adipose tissue of British Columbia residents. *Can. J. Public Health*, **84**(3): 192-196.

THAO, V.D., KAWANO, M. AND TATSUKAWA, R., 1993. Persistent organochlorine residues in soils from tropical and sub-tropical Asian countries. *Environ. Pollut.*, **81**(1): 61-71.

THORPE, E. AND WALKER, A.I.T., 1973. The toxicity of dieldrin (HEOD). II. Comparative long term oral toxicity studies in mice with dieldrin, DDT, phenoharbitone, *beta*-BHC and *gamma*-BHC. *Food Cosmet. Toxicol.*, **11**: 433-442.

TURUSOV, V.S., DAY, N.E., TOATIS, L., GATI, E. AND CHARLES, R.T., 1973. Tumours in CF-1 mice exposed for 6 consecutive generations to DDT. *J. Natl. Cancer Inst.*, **51**: 983-997.

URDANETA, H., MEDINA, B. AND ACOSTA, Z., 1995. Organochlorine compounds in fish from a farming station in the Municipality of Paez, state of Zulia, Venezuella. *Bull. Environ. Contam. Toxicol.*, **54**: 703-710.

VUORINEN, P.J., RANTIO, T., WITICK, A. AND VUORINEN, M., 1994. Organochlorines and heavy metals in sea trout (*Salmo trutta* M.) in the Gulf of Bothnia off the coast of Finland. *Aqua Fenn.*, **24**(1): 29-35.

WINTER, S. AND STREET, B., 1992. Organochlorine compounds in the three step terrestrial food chain. *Chemosphere*, **24**: 1765-1774.

YUSOF, Y.A.M. AND EDWARDS, A.M., 1990. Stimulation of DNA synthesis in primary rat hepatocytes cultures by liver tumor promotors: Interactions with other growth factors. *Carcinogenesis*, **11**(5): 761-770.

(Received: April 18, 1996)