



Toxicity and Carcinogenicity of Dichlorodiphenyltrichloroethane (DDT)

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Dichlorodiphenyltrichloroethane (DDT) is still used in certain areas of tropics and subtropics to control malaria and other insect-transmitted diseases. DDT and its metabolites have been extensively studied for their toxicity and carcinogenicity in animals and humans and shown to have an endocrine disrupting potential affecting reproductive system although the effects may vary among animal species in correlation with exposure levels. Epidemiologic studies revealed either positive or negative associations between exposure to DDT and tumor development, but there has been no clear evidence that DDT causes cancer in humans. In experimental animals, tumor induction by DDT has been shown in the liver, lung, and adrenals. The mechanisms of hepatic tumor development by DDT have been studied in rats and mice. DDT is known as a non-genotoxic hepatocarcinogen and has been shown to induce microsomal enzymes through activation of constitutive androstane receptor (CAR) and to inhibit gap junctional intercellular communication (GJIC) in the rodent liver. The results from our previously conducted 4-week and 2-year feeding studies of *p,p'*-DDT in F344 rats indicate that DDT may induce hepatocellular eosinophilic foci as a result of oxidative DNA damage and leads them to hepatic neoplasia in combination with its mitogenic activity and inhibitory effect on GJIC. Oxidative stress could be a key factor in hepatocarcinogenesis by DDT.

Key words: Enzyme induction, CAR activation, Oxidative stress, Cell proliferation, Intercellular communication, Eosinophilic foci, DDT

INTRODUCTION

DDT (dichlorodiphenyltrichloroethane) was first synthesized in 1874 and its insecticidal properties were discovered in 1939. Since then DDT was widely used in the world to control insects on agricultural crops and those that carry diseases such as malaria and typhus. However, the use of this compound has been banned in many countries since 1970s because of its chemical characteristics such as accumulation and bio-concentration in lipid systems of all animal species which may result in occurrence of potential adverse effects on humans and wild animals (1). DDT has been suggested to be toxic to a range of wildlife including birds and marine animals, and its metabolite DDE (dichlorodiphenyldichloroethylene) causes eggshell thinning of

certain bird species such as bald eagle and brown pelican, leading to declines of their populations (1,2). It is considered that DDE inhibits calcium adenosine triphosphatase (ATPase) in the membrane of the shell gland and reduces the transport of calcium carbonate from blood into the eggshell gland (1). Despite these circumstances, DDT is still used in the certain areas of tropics and subtropics for the control of malaria and other insect-transmitted diseases causing high death rates (3). In 2006, World Health Organization (WHO) permitted the use of DDT in those areas to reduce the rate of deaths caused by malaria. DDT and its metabolites have been extensively studied for their toxicity and carcinogenicity in experimental animals and humans and the results of their investigations are well documented (1,4). It has been shown that DDT and its metabolites may have adverse effects on various organs/tissues of mammals including nervous, liver, kidney, reproductive, endocrine, and immune systems (1,4). The present review paper describes the overview of neurotoxicity, reproductive toxicity with endocrine effects, hepatotoxicity and carcinogenicity of DDT and its metabolites. In addition, potential factors including microsomal enzymes, cell proliferation, intercellular communication, oxidative stress which might be involved in hepatocarcinogenesis by DDT are discussed based on the

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results of our previously conducted 4-week and 2-year feeding studies of *p,p'*-DDT in F344 rats (5). Furthermore, a potential effect of DDT or its metabolites on mitochondria is also addressed, since the mitochondrion plays a crucial role in maintaining hepatocyte integrity and functions, and the mitochondrial dysfunction is considered one of the important mechanisms for the chemical-mediated hepatic toxicity and/or carcinogenicity (6-9).

Neurotoxicity. It is known that DDT delays the closing of the sodium ion channel and prevents the opening of the potassium gates and also targets a specific neuronal ATPase considered to be involved in the control of the rate of sodium, potassium, and calcium fluxes through the nerve membrane (1). In addition, DDT has been suggested to inhibit the ability to transport calcium ions which are essential to the release of neurotransmitters. These actions combine to effectively maintain the depolarization of the nerve membrane, potentiating the release of transmitters and leading to central nervous system excitation manifested as hyperexcitability, tremors, and convulsions. It was reported that occupational exposure to DDT in retired workers from Costa Rica was associated with neurobehavioral symptoms in a dose-response pattern (10). In our 2-year rat feeding study of *p,p'*-DDT, a whole body tremor was observed in the high dose (500 ppm) group of both sexes that was more evident in females. This sex difference was consistent with the toxicokinetics data obtained from the rat 2-year study in that plasma and brain concentrations of DDT and its metabolite DDE tended to be higher in females than males (Figs. 1 and 2).

Reproductive toxicity with endocrine effects. It has been suggested that DDT and its metabolites may have an endocrine disrupting potential to affect reproductive system through their estrogenic or androgenic activity (1). Receptor-binding assays indicate that *o,p'*-DDT has weak estrogenic activity and *p,p'*-DDE is an androgen receptor

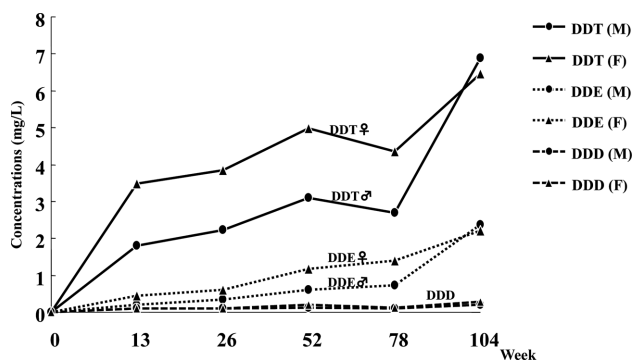


Fig. 1. Plasma concentration of DDT, DDE, and DDD in F344 rats at high-dose (500 ppm) in a 2-year feeding study. (M) male; (F) female.

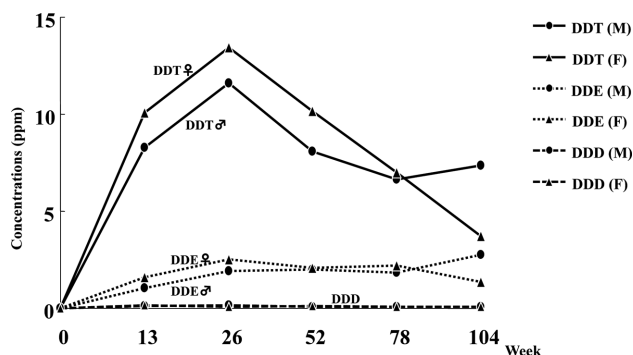


Fig. 2. Brain concentration of DDT, DDE, and DDD in F344 rats at high-dose (500 ppm) in a 2-year feeding study. (M) male; (F) female.

antagonist, while the main DDT's component *p,p'*-DDT has little estrogenic or androgenic activity (11). In our institute, a 2-generation reproduction toxicity study of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p'*-DDT) in Sprague Dawley (SD) rats was conducted in accordance with the current test guidelines of MAFF (Japan), EPA (USA) and OECD with some modifications and additions (12). In the reproduction study, *p,p'*-DDT was given to parental rats at dietary levels of 0, 5, 50 or 350 ppm. As a result, parental animals in the mid- and high-dose groups showed tremors (both sexes), subsequent deaths (females only), and/or pathological changes in the liver (both sexes). However, reproductive and postnatal developmental toxicities were not evident up to 350 ppm (high-dose) except for the decreased pup viability index on postnatal day 21 in the high-dose group. Although changes in serum estradiol and progesterone levels and/or a delay in male sexual maturation were noted in the mid- and high-dose groups, no substantial reproductive disorders occurred. Based on these results, it was concluded that the *in vivo* effects of *p,p'*-DDT associated with its endocrine activities are not adverse at least under the experimental condition and thus *p,p'*-DDT is not considered to exert overt endocrine disrupting effects in SD rats at levels up to 350 ppm. The reproductive toxicity with endocrine effects of DDT may differ among animal species/strains, depending on their susceptibility and dose levels.

Hepatotoxicity and carcinogenicity. Hepatotoxicity and carcinogenicity of DDT have been demonstrated in animals to show increased liver weights, hepatocellular hypertrophy, microsomal enzyme induction similar to phenobarbital, and hepatocellular tumor induction (1). Many epidemiologic studies have been conducted and the results indicate either positive or negative associations between exposure to DDT and tumor development in humans (1,13). The International Agency for Research on Cancer (IARC) classifies DDT into Group 2B (possibly carcinogenic to humans). However, there has been no clear evidence that exposure to

DDT causes cancer in humans. The toxic changes caused by DDT in the liver and potential factors involved in hepatocarcinogenesis by DDT are discussed in the following sections on the basis of the results obtained from our previously conducted 4-week feeding study of *p,p'*-DDT in male F344 rats at 0, 50, 160, 500 ppm and 2-year feeding study of *p,p'*-DDT in F344 rats of both sexes at 0, 5, 50, 500 ppm (5).

Hepatic microsomal enzyme induction. Analyses of hepatic microsomal enzymes revealed significant increases in pentoxoresorufin O-dealkylase (PROD) activity and P450 isozyme contents of CYP2B1 and CYP3A2 in the DDT-treated rats (Tables 1~3). The results are generally consistent with previous works (14-17,23-25) and considered due to activation of constitutive androstane receptor (CAR) (24). As shown in Tables 1~3, the increases in CYP2B1 and CYP3A2 were dose-dependent, whereas the elevation of PROD activity was most evident in the mid-dose group and

not significant in males of the high-dose group after 52, 78, and 104 weeks. This suggests that microsomal enzyme activity is not always consistent with its associated protein content and there seems to be an almost inverse correlation between the increase in PROD at different time points and the concurrent incidence of pre-neoplastic hepatocellular eosinophilic foci and hepatocellular tumors. As to P450 isozyme contents, the increases in CYP2B1 and CYP3A2 tended to be more evident in females than males. A similar result (preferential induction of CYP3A2 in females) also has been reported in Wistar rats treated with the technical grade DDT, a mixture of *p,p'*-DDT (85%), *o,p'*-DDT (15%) and *o,o'*-DDT (trace amount) (17). Since CYP3A2 is androgen-dependent and not normally expressed in adult female rats (25), the induction of CYP3A2 by DDT in female rats suggests that DDT is able to modulate sexual metabolic dimorphism by affecting regulatory sites of hepatic metabolism (17). The preferential induction of CYP2B1 and CYP3A2 by DDT in female rats indicates an endocrine dis-

Table 1. Hepatic microsomal P450 isozyme content in male F344 rats from a 4-week feeding study of *p,p'*-DDT

Dose (ppm)	CYP2B1 content (pmol/mg protein) on days:					
	1	2	3	7	14	28
0	20.01 ± 5.41	12.95 ± 1.23	11.93 ± 2.93	12.97 ± 2.77	12.54 ± 1.73	9.47 ± 2.41
50	96.13 ± 12.70**	187.10 ± 17.52**	181.06 ± 52.39**	210.41 ± 25.13**	185.19 ± 36.17**	273.32 ± 48.78**
160	162.45 ± 19.27**	321.44 ± 33.67**	248.15 ± 27.01**	244.15 ± 33.03**	329.74 ± 63.64**	252.10 ± 31.65**
500	166.65 ± 53.76**	274.82 ± 21.13**	281.66 ± 45.10**	280.96 ± 53.66**	303.18 ± 21.92**	348.87 ± 80.59**

Values represent mean ± S.D. (n = 5).

** : Significantly different from control at $p < 0.01$ (Dunnett's multiple comparison test).

Table 2. Hepatic microsomal enzyme activity and P450 isozyme contents in male F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	PROD (pmol/min/mg)	P450 isozyme contents (pmol/mg protein)								
			CYP1A2		CYP2B1		CYP3A2		CYP4A1		
0	26	18 ± 2	(6)	4.24 ± 0.97	(6)	10.4 ± 1.0	(6)	72.3 ± 15.3	(6)	21.9 ± 2.1	(6)
	52	15 ± 2	(6)	3.22 ± 0.97	(6)	9.75 ± 1.02	(6)	83.4 ± 6.9	(6)	35.4 ± 2.8	(6)
	78	5 ± 2	(6)	6.97 ± 1.42	(6)	11.4 ± 1.7	(6)	69.6 ± 16.3	(6)	40.9 ± 7.5	(6)
	104	3 ± 2	(6)	6.37 ± 1.90	(6)	12.4 ± 2.7	(6)	10.1 ± 5.9	(6)	17.5 ± 4.7	(6)
5	26	155 ± 9	(6)	6.88 ± 1.39**	(6)	66.7 ± 8.9	(6)	175 ± 23**	(6)	19.4 ± 1.6	(6)
	52	205 ± 18**	(6)	7.05 ± 1.15**	(6)	61.9 ± 8.0	(6)	175 ± 18	(6)	36.3 ± 5.4	(6)
	78	118 ± 15*	(6)	5.55 ± 1.50	(6)	74.9 ± 9.0	(6)	94.0 ± 36.0	(6)	39.1 ± 12.4	(6)
	104	81 ± 29*	(6)	8.99 ± 4.39	(6)	76.2 ± 17.8	(6)	56.6 ± 31.6	(6)	18.4 ± 7.7	(6)
50	26	456 ± 25**	(6)	3.25 ± 0.70	(6)	366 ± 34**	(6)	368 ± 34**	(6)	35.8 ± 2.4**	(6)
	52	466 ± 42**	(6)	2.65 ± 1.19	(6)	398 ± 50**	(6)	364 ± 56**	(6)	44.4 ± 7.0*	(6)
	78	293 ± 95**	(6)	3.44 ± 3.06*	(6)	172 ± 56**	(6)	150 ± 82	(6)	42.0 ± 15.8	(6)
	104	143 ± 45**	(6)	7.79 ± 4.04	(6)	179 ± 33**	(6)	60.4 ± 16.4	(6)	20.4 ± 8.7	(6)
500	26	173 ± 26*	(6)	1.78 ± 0.36**	(6)	607 ± 71**	(6)	534 ± 50**	(6)	36.5 ± 3.9**	(6)
	52	143 ± 15	(6)	3.96 ± 0.87	(6)	476 ± 70**	(6)	540 ± 92**	(6)	33.4 ± 3.3	(6)
	78	81 ± 15	(6)	3.73 ± 2.64	(6)	222 ± 38**	(6)	189 ± 74**	(6)	30.4 ± 6.2	(6)
	104	64 ± 26	(6)	5.04 ± 1.62	(6)	271 ± 65**	(6)	176 ± 42**	(6)	18.8 ± 9.1	(6)

Values represent mean ± S.D.

(n): Number of animals examined.

*** : Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 3. Hepatic microsomal enzyme activity and P450 isozyme contents in female F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	PROD (pmol/min/mg)	P450 isozyme contents (pmol/mg protein)								
			CYP1A2		CYP2B1		CYP3A2		CYP4A1		
0	26	12 ± 1	(6)	16.8 ± 6.5	(6)	6.48 ± 0.94	(6)	10.7 ± 1.7	(6)	18.8 ± 2.5	(6)
	52	8 ± 2	(6)	21.6 ± 6.2	(6)	3.81 ± 1.11	(6)	12.7 ± 2.1	(6)	14.4 ± 2.0	(6)
	78	7 ± 1	(6)	10.9 ± 2.9	(6)	4.48 ± 0.93	(6)	12.1 ± 2.7	(6)	20.7 ± 5.1	(6)
	104	4 ± 3	(6)	17.9 ± 8.2	(6)	5.86 ± 0.77	(6)	7.98 ± 5.28	(6)	17.9 ± 5.4	(6)
5	26	92 ± 16	(6)	22.6 ± 4.3	(6)	32.5 ± 3.6	(6)	16.4 ± 1.7	(6)	15.8 ± 2.2	(6)
	52	95 ± 26	(6)	29.6 ± 9.9	(6)	16.9 ± 4.6	(6)	25.8 ± 4.4	(6)	20.1 ± 1.3**	(6)
	78	51 ± 13	(6)	12.0 ± 3.5	(6)	37.4 ± 3.7	(6)	29.2 ± 5.2	(6)	19.2 ± 4.7	(6)
	104	50 ± 18	(6)	17.9 ± 4.8	(6)	23.8 ± 6.3	(6)	28.6 ± 8.4	(6)	22.2 ± 6.5	(6)
50	26	402 ± 74**	(6)	23.6 ± 2.4	(6)	263 ± 39**	(6)	53.5 ± 9.8**	(6)	19.5 ± 2.9	(6)
	52	393 ± 44**	(6)	31.3 ± 6.2	(6)	157 ± 17**	(6)	82.3 ± 8.9**	(6)	26.1 ± 3.0**	(6)
	78	237 ± 23**	(6)	11.5 ± 4.5	(6)	183 ± 39**	(6)	80.8 ± 12.4**	(6)	21.4 ± 0.5	(6)
	104	171 ± 39**	(6)	14.5 ± 4.0	(6)	104 ± 25**	(6)	63.1 ± 11.3**	(6)	21.4 ± 1.7	(6)
500	26	245 ± 44**	(6)	20.6 ± 4.4	(6)	371 ± 46**	(6)	171 ± 25**	(6)	14.8 ± 4.3	(6)
	52	241 ± 30**	(6)	31.0 ± 7.2	(6)	350 ± 29**	(6)	155 ± 21**	(6)	20.3 ± 2.9**	(6)
	78	106 ± 10**	(6)	18.8 ± 3.5**	(6)	218 ± 29**	(6)	171 ± 35**	(6)	15.7 ± 3.5	(6)
	104	90 ± 25*	(5)	20.6 ± 1.5	(5)	210 ± 34**	(5)	189 ± 28**	(5)	15.6 ± 4.8	(5)

Values represent mean ± S.D.

(n): Number of animals examined.

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

rupting potential of DDT because these CYPs are involved in steroid metabolism. With respect to other P450 isozyme contents, statistically significant increases or decreases in CYP1A2 and CYP4A1 were noted in the DDT-treated groups of both sexes, but those changes were not consistent during the study and their toxicological significance remains obscure.

Oxidative stress. Measurements of hepatic oxidative stress markers in our 2-year rat study disclosed significant increases in lipid peroxide (LPO) and 8-hydroxydeoxyguanosine (8-OHdG) in the mid- and high-dose groups that developed hepatocellular tumors, which were more evident in males than females (Tables 4 and 5). These results indicate that hepatocytes in the DDT-treated livers are exposed

to oxidative stress and could have cellular and DNA damages. It is postulated that the metabolic activation with enzyme induction of P450 by DDT may result in formation of reactive oxygen radicals (23,26). In addition, it is conceivable that DDT or its metabolites may affect mitochondria and cause mitochondrial dysfunction which results in formation of deleterious reactive oxygen species (ROS) leading to lipid peroxidation since mitochondria are prominent target sites of many hepatotoxic chemicals and also the main source of ROS in the hepatocytes (6-9). In the DDT-treated groups that developed hepatocellular carcinomas, 8-OHdG levels were significantly increased. The increase in 8-OHdG levels (an evidence of oxidative DNA damage) could play an important role in hepatocarcinogenesis by DDT. It has been shown that 8-OHdG leads to base mis-

Table 4. Hepatic lipid peroxide (LPO) contents in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Sex	No. of rats examined	LPO (nmol/g tissue) at weeks			
			26	52	78	104
0	M	6	130 ± 13	152 ± 37	157 ± 37	138 ± 29
	F	6	168 ± 30	202 ± 15	150 ± 21	173 ± 53
5	M	6	210 ± 113	140 ± 30	217 ± 71	257 ± 122
	F	6	177 ± 20	188 ± 8	157 ± 43	188 ± 77
50	M	6	1005 ± 324**	537 ± 74**	530 ± 132**	523 ± 332*
	F	6	247 ± 62*	248 ± 75	212 ± 48	307 ± 78*
500	M	6	838 ± 286**	393 ± 54**	360 ± 172*	423 ± 68**
	F	6	267 ± 69*	178 ± 24	185 ± 53	267 ± 101

Values represent mean ± S.D.

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 5. Hepatic 8-OHdG levels in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Sex	No. of rats examined	8-OHdG (mg/mg DNA) at weeks			
			26	52	78	104
0	M	6	4.4 ± 1.0	3.0 ± 0.9	2.7 ± 1.1	3.2 ± 0.8
	F	6	1.9 ± 0.5	2.7 ± 0.5	2.5 ± 0.5	3.0 ± 0.8
5	M	6	3.7 ± 1.3	3.8 ± 0.8	2.2 ± 0.7	3.5 ± 0.7
	F	6	2.0 ± 0.2	4.0 ± 1.9	2.7 ± 0.3	2.9 ± 0.6
50	M	6	5.9 ± 1.6	6.1 ± 2.8	2.9 ± 1.2	5.0 ± 1.0
	F	6	3.0 ± 1.6	5.2 ± 1.4	3.1 ± 0.4	2.7 ± 0.5
500	M	6	15.4 ± 4.5**	13.8 ± 4.3**	7.5 ± 2.2**	15.9 ± 9.9**
	F	6	2.6 ± 1.1	14.1 ± 5.2**	6.2 ± 2.9**	5.8 ± 0.9**

Values represent mean ± S.D.

** : Significantly different from control at $p < 0.01$ (Dunnett's multiple comparison test).

pairing (mutation) on DNA replication (27). In our 2-year rat study, the appearance of eosinophilic altered hepatocellular foci (AHF) in the high-dose group was significantly earlier than that in controls. This result indicates that the occurrence of initiated cells could be accelerated by DDT in the high-dose group, which is considered to be due to hepatocellular DNA damage caused by oxidative stress. It is possible that the accelerated occurrence of initiated cells may result in the early appearance and increased incidence of eosinophilic AHF described later.

Cell proliferation. PCNA labeling index (LI) in the liver from rats treated with DDT showed that cell proliferation was enhanced within 3 days of treatment but returned

to normal thereafter (Tables 6 and 7). This cell proliferation pattern is consistent with that by non-genotoxic mitogenic hepatocarcinogens (28-30). It is generally known that the hepatic cell proliferation response to non-genotoxic mitogenic hepatocarcinogens such as phenobarbital typically occurs through an initial burst of enhanced DNA synthesis followed by enhanced mitosis (29-31). The enhanced cell proliferation, however, ceases after a few days even if treatment is continued. It is considered that an effective feedback mechanism (checkpoint function such as G1 or G2 arrest in cell cycle) may prevent excessive cell multiplication in the normal liver even if the growth stimulatory signals are steadily present due to continuous chemical treatment (30). It has been postulated that non-genotoxic chemicals

Table 6. PCNA LI (%) in the liver of male F344 rats from a 4-week feeding study of *p,p'*-DDT

Dose (ppm)	PCNA LI (mean ± SD) on days									
	1	2	3	7	14	28				
0	2.00 ± 0.80 (5)	1.58 ± 0.68 (5)	3.19 ± 1.44 (5)	4.46 ± 1.41 (5)	1.75 ± 0.77 (5)	0.96 ± 0.50 (5)				
50	2.06 ± 0.46 (5)	3.22 ± 0.51* (5)	4.84 ± 1.60 (5)	5.37 ± 0.36 (5)	0.96 ± 0.31 (5)	0.58 ± 0.18 (5)				
160	2.81 ± 0.67 (5)	5.91 ± 1.49** (5)	6.85 ± 1.71** (5)	4.13 ± 0.72 (5)	0.62 ± 0.54 (5)	0.60 ± 0.34 (5)				
500	4.62 ± 1.01** (5)	5.33 ± 0.59** (5)	6.31 ± 1.57* (5)	4.52 ± 1.27 (5)	1.39 ± 1.19 (5)	0.94 ± 0.48 (5)				

(n): Number of animals examined.

** : Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 7. PCNA LI (%) in the liver of F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Sex	PCNA LI (mean ± SD) at weeks			
		26	52	78	104
0	M	0.70 ± 0.34 (6)	0.43 ± 0.11 (6)	0.82 ± 0.63 (6)	0.80 ± 1.03 (5)
	F	0.25 ± 0.22 (6)	0.17 ± 0.11 (6)	0.93 ± 0.52 (6)	0.45 ± 0.32 (5)
5	M	0.67 ± 0.38 (6)	0.40 ± 0.22 (6)	0.69 ± 0.25 (6)	0.43 ± 0.19 (5)
	F	0.13 ± 0.05 (6)	0.14 ± 0.08 (6)	1.13 ± 1.17 (6)	0.28 ± 0.07 (6)
50	M	0.77 ± 0.19 (6)	0.28 ± 0.13 (6)	0.75 ± 0.31 (6)	0.42 ± 0.21 (4)
	F	0.87 ± 0.69 (6)	0.36 ± 0.16 (6)	1.10 ± 0.59 (6)	0.46 ± 0.25 (6)
500	M	0.32 ± 0.22 (6)	0.29 ± 0.16 (6)	0.78 ± 0.24 (6)	0.73 ± 0.48 (6)
	F	0.45 ± 0.18 (6)	0.34 ± 0.31 (6)	0.62 ± 0.20 (6)	0.55 ± 0.06 (4)

(n): Number of animals examined.

with mitogenic activity may provide a selective growth advantage to spontaneously initiated precancerous cells over normal hepatocytes and lead them to neoplasms (28,29). The increases in the number and size of eosinophilic AHF in our 2-year study could be a reflection of the mitogenic activity of DDT that contributes to the growth of initiated cells.

Gap junctional intercellular communication (GJIC).

Quantitative analysis of gap junctional intercellular communication (GJIC) in the liver from rats treated with DDT demonstrated a persistent decrease in GJIC protein Cx32 from the beginning to the end of treatment (Tables 8 and 9). GJIC in the liver has been shown to be inhibited by various

non-genotoxic tumor-promoting agents including phenobarbital and DDT in vivo and in vitro (14,18-20,22,32-34). The inhibition of GJIC by tumor promoters may be produced in several ways (21). Since DDT is highly lipophilic and accumulates in cell membranes, it could interfere directly with the function of GJIC, whereas phenobarbital which is not highly lipophilic may inhibit GJIC in a different way. It is known that GJIC involves the passage of low molecular weight substances between adjacent cells via gap junctions and its function includes the possible regulation of cellular division through cell-to-cell exchange of replication signal molecules (19,34,35). Therefore, inhibition of GJIC may isolate initiated cells from the growth regulatory signals of neighboring cells and permit the clonal expansion of initi-

Table 8. Number of GJIC protein Cx32 spots in the liver of male F344 rats from a 4-week feeding study of *p,p'*-DDT

Dose (ppm)	No. of Cx32 spots (mean ± SD) on days:											
	1		2		3		7		14		28	
0	8.29 ± 0.36	(5)	7.81 ± 0.75	(5)	7.86 ± 0.81	(5)	7.24 ± 0.38	(5)	7.64 ± 0.52	(5)	8.11 ± 0.22	(5)
50	5.59 ± 0.77**	(5)	4.69 ± 0.33**	(5)	5.41 ± 0.40**	(5)	3.14 ± 0.33**	(5)	2.99 ± 0.53**	(5)	3.33 ± 0.74**	(5)
160	4.90 ± 0.63**	(5)	4.37 ± 0.64**	(5)	3.94 ± 0.64**	(5)	3.06 ± 0.46**	(5)	2.71 ± 0.68**	(5)	2.77 ± 0.43**	(5)
500	5.35 ± 1.03**	(5)	3.98 ± 0.41**	(5)	3.12 ± 0.33**	(5)	2.62 ± 0.70**	(5)	1.75 ± 0.51**	(5)	1.68 ± 0.79**	(5)

(n): Number of animals examined.

** : Significantly different from control at $p < 0.01$ (Dunnett's multiple comparison test).

Table 9. Number of GJIC protein Cx32 spots in the liver of F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Sex	No. of Cx32 spots (mean ± SD) at weeks							
		26		52		78		104	
0	M	7.07 ± 0.94	(6)	6.49 ± 1.46	(6)	3.05 ± 0.19	(6)	3.11 ± 1.15	(5)
	F	6.08 ± 0.97	(6)	6.48 ± 0.66	(6)	3.26 ± 0.74	(6)	3.73 ± 0.23	(5)
5	M	6.87 ± 0.54	(6)	6.95 ± 0.88	(6)	2.51 ± 0.50*	(6)	2.13 ± 0.37	(5)
	F	5.67 ± 0.93	(6)	5.51 ± 1.62	(6)	2.90 ± 0.41	(6)	3.59 ± 0.42	(6)
50	M	5.53 ± 0.83*	(6)	6.28 ± 1.88	(6)	2.47 ± 0.44*	(6)	2.13 ± 0.35	(4)
	F	4.80 ± 0.86	(6)	4.20 ± 0.56**	(6)	1.76 ± 0.69*	(6)	2.65 ± 0.33**	(6)
500	M	5.39 ± 1.30*	(6)	4.64 ± 0.91	(6)	1.14 ± 0.22**	(6)	1.52 ± 0.28*	(6)
	F	4.20 ± 1.41*	(6)	3.28 ± 0.72**	(6)	1.03 ± 0.16**	(6)	1.75 ± 0.47**	(4)

(n): Number of animals examined.

*** : Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 10. Liver weights of male F344 rats from a 4-week feeding study of *p,p'*-DDT

Dose (ppm)	Liver weights	Days on study					
		1	2	3	7	14	28
50	Absolute	109 (5)	107 (5)	106 (5)	112 (5)*	126 (5)*	112 (5)**
	Relative	108 (5)**	107 (5)	107 (5)*	111 (5)*	121 (5)**	110 (5)**
160	Absolute	105 (5)	114 (5)	121 (5)**	127 (5)**	142 (5)**	124 (5)**
	Relative	107 (5)*	113 (5)**	119 (5)**	123 (5)**	136 (5)**	122 (5)**
500	Absolute	110 (5)	125 (5)**	127 (5)**	153 (5)**	148 (5)**	142 (5)**
	Relative	111 (5)**	125 (5)**	129 (5)**	147 (5)**	148 (5)**	145 (5)**

Values represent percentages (%) to control values.

(n): Number of animals examined.

Relative: Ratio to body weight (%)

*** : Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 11. Liver weights of male F344 rats from a 2-year feeding study of p,p' -DDT

Dose (ppm)	Liver weights	Weeks on study			
		26	52	78	104
5	Absolute	106 (6)	108 (6)	108 (8)	110 (10)
	Relative	102 (6)	108 (6)	105 (8)	105 (10)
50	Absolute	114 (6)**	117 (6)*	126 (8)**	125 (10)**
	Relative	112 (6)*	118 (6)*	125 (8)**	123 (10)*
500	Absolute	166 (6)**	154 (6)**	153 (7)**	149 (10)**
	Relative	170 (6)**	168 (6)**	166 (7)**	171 (10)**

Values represent percentages (%) to control values.

(n): Number of animals examined.

Relative: Ratio to body weight (%)

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

Table 12. Liver weights of female F344 rats from a 2-year feeding study of p,p' -DDT

Dose (ppm)	Liver weights	Weeks on study			
		26	52	78	104
5	Absolute	109 (6)	106 (6)	104 (8)	102 (10)
	Relative	106 (6)	103 (6)	103 (8)	101 (10)
50	Absolute	111 (6)*	123 (6)	110 (7)	111 (10)
	Relative	114 (6)*	118 (6)**	109 (7)	110 (10)
500	Absolute	146 (6)**	151 (6)**	143 (8)**	144 (10)**
	Relative	163 (6)**	170 (6)**	165 (8)**	190 (10)**

Values represent percentages (%) to control values.

(n): Number of animals examined.

Relative: Ratio to body weight (%)

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's multiple comparison test).

ated cells. This suggests an important role for GJIC in the process of tumor formation.

Changes in liver weights. Time-related changes in liver weights in F344 rats treated with DDT are shown in Tables 10–12. Absolute and/or relative (ratio to body weight) liver weights significantly increased in a dose-dependent manner from the beginning of treatment throughout the study. The liver weight also increased in correlation with duration of exposure but tended to reach a plateau after certain time. It is known that administration of mitogenic agents such as phenobarbital and buthylhydroxytoluene causes an increase in liver weight through mitogenic stimulation of cell proliferation (28). Upon continued administration, the increased liver weight is maintained, even though the rate of cell turnover returns to normal levels (28). The increased liver weights in the DDT-treated rats might be due to mitogenic stimulation of cell proliferation to some extent, but the major cause seems to be proliferation of smooth-surfaced endoplasmic reticulum (SER) associated with microsomal enzyme induction since the time of increase in liver weight and enzyme induction was consistent with each other during the study.

Histopathology of the liver with morphometry. In our 2-year rat study, treatment with DDT induced centrilob-

ular hepatocellular hypertrophy and increased eosinophilic AHF, hepatocellular adenomas and carcinomas (Table 13). The severity of hepatocellular hypertrophy (Fig. 4A) was dose-dependent and the hypertrophic alteration could be due to SER proliferation associated with microsomal enzyme induction. The eosinophilic AHF noted in the liver treated with DDT typically contained hepatocytes with pale pink or ground glass appearance cytoplasm (Fig. 4B) and sometimes had cytoplasmic clear spaces which may represent glycogen deposits. The eosinophilic AHF was positive for glutathione S-transferase placental form (GST-P) (Fig. 4C). The eosinophilic AHF tended to be located in the region close to or within the hypertrophic area. Such a spatial relationship was also reported in rats after continuous administration of low doses of N-nitrosomorpholine, which resulted in centrilobular perivenular hepatocellular hypertrophy, being closely related to the later development of pre-neoplastic hepatic foci including clear/acidophilic foci as well as hepatocellular neoplasms (36).

Quantitative morphometric analysis revealed that the number and size of eosinophilic AHF were increased in correlation with duration of exposure and dose levels and its appearance was earlier in males than females (Tables 14–16). The incidence of eosinophilic AHF in the high-dose group after 104 weeks of treatment reached nearly 100%

Table 13. Incidences of hepatocellular hypertrophy and altered hepatocellular foci (AHF) in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	Hypertrophy		Eosinophilic AHF		Tigroid basophilic AHF		Clear cell AHF	
		Male	Female	Male	Female	Male	Female	Male	Female
0	26	0/6	0/6	0/6	0/6	2/6	2/6	0/6	0/6
	52	0/6	0/6	0/6	0/6	3/6	4/6	5/6	0/6
	78	0/8	0/8	1/8	0/8	8/8	8/8	5/8	3/8
	104	0/35	0/33	23/35	6/33	34/35	30/33	30/35	8/33
	Total ^a	0/40	0/40	24/40	8/40	37/40	37/40	30/40	9/40
5	26	2/6	0/6	0/6	0/6	1/6	1/6	1/6	0/6
	52	6/6**	0/6	0/6	0/6	1/6	6/6	4/6	0/6
	78	4/8*	1/8	2/8	1/8	8/8	8/8	6/8	2/8
	104	15/30**	1/27	18/30	8/27	29/30	26/27	24/30	0/27**
	Total	15/40**	1/40	21/40	10/40	35/40	38/40	24/40	0/40**
50	26	6/6**	6/6**	0/6	0/6	1/6	3/6	1/6	0/6
	52	6/6**	6/6**	0/6	0/6	0/6	6/6	4/6	0/6
	78	8/8**	7/7**	8/8**	1/7	8/8	7/7	8/8	0/7
	104	33/36**	31/32**	30/36	16/32**	34/36	30/32	27/36	8/32
	Total	35/40**	34/40**	34/40**	19/40**	38/40	38/40	27/40	8/40
500	26	6/6**	6/6**	4/6*	0/6	0/6	2/6	0/6	0/6
	52	6/6**	6/6**	6/6**	2/6	0/6	0/6*	0/6**	0/6
	78	7/7**	8/8**	7/7**	8/8**	3/7*	6/8	0/7**	0/8
	104	31/33**	31/33**	33/33**	31/33**	29/33	23/33*	13/33**	11/33
	Total	38/40**	37/40**	38/40**	37/40**	34/40	27/40**	14/40*	11/40

^a: Total number of animals examined (No. of scheduled deaths after 104 weeks + No. of unscheduled deaths).

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Fisher's exact test).

Table 14. Number of altered hepatocellular foci (AHF) in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	No. of rats examined		No. of AHF (No./cm ²): Mean \pm SD					
				Eosinophilic		Tigroid basophilic		Clear cell	
		Male	Female	Male	Female	Male	Female	Male	Female
0	26	6	6	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.6	0.6 \pm 0.9	0.0 \pm 0.0	0.0 \pm 0.0
	52	6	6	0.0 \pm 0.0	0.0 \pm 0.0	1.0 \pm 1.3	1.6 \pm 1.6	1.6 \pm 1.1	0.0 \pm 0.0
	78	8	8	0.1 \pm 0.4	0.0 \pm 0.0	2.2 \pm 1.3	9.3 \pm 3.7	0.8 \pm 0.8	0.5 \pm 0.7
	104	10	10	1.5 \pm 1.5	0.6 \pm 1.0	6.5 \pm 4.8	11.0 \pm 5.3	1.9 \pm 1.7	0.8 \pm 0.9
5	26	6	6	0.0 \pm 0.0	0.0 \pm 0.0	0.2 \pm 0.4	0.3 \pm 0.7	0.2 \pm 0.4	0.0 \pm 0.0
	52	6	6	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.8	4.7 \pm 1.7	1.3 \pm 1.4	0.0 \pm 0.0
	78	8	8	0.5 \pm 0.9	0.2 \pm 0.5	2.8 \pm 1.7	10.0 \pm 5.3	1.4 \pm 1.2	0.6 \pm 1.1
	104	10	10	2.0 \pm 1.8	0.6 \pm 0.7	5.1 \pm 4.6	14.2 \pm 4.0	2.4 \pm 2.0	0.0 \pm 0.0
50	26	6	6	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.9	0.8 \pm 0.8	0.2 \pm 0.4	0.0 \pm 0.0
	52	6	6	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	5.5 \pm 1.6*	1.1 \pm 1.1	0.0 \pm 0.0
	78	8	7	3.1 \pm 1.5*	0.6 \pm 1.5	2.0 \pm 1.9	8.7 \pm 4.1	1.2 \pm 0.5	0.0 \pm 0.0
	104	10	10	8.9 \pm 5.1**	1.0 \pm 2.2	3.8 \pm 2.3	17.6 \pm 4.5	2.9 \pm 1.2	0.3 \pm 0.7
500	26	6	6	0.8 \pm 0.7**	0.0 \pm 0.0	0.0 \pm 0.0	0.5 \pm 0.8	0.0 \pm 0.0	0.0 \pm 0.0
	52	6	6	3.7 \pm 1.6**	1.4 \pm 2.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0*	0.0 \pm 0.0
	78	7	8	9.2 \pm 3.1**	12.1 \pm 3.5**	0.5 \pm 0.6	1.0 \pm 0.7**	0.0 \pm 0.0	0.0 \pm 0.0
	104	10	10	10.4 \pm 3.1**	9.8 \pm 3.8**	2.1 \pm 1.0	1.3 \pm 1.3*	0.4 \pm 0.6*	1.0 \pm 1.3

***: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Fisher's exact test).

for both sexes. Since the first appearance of eosinophilic AHF in the high-dose group (at week 26) was much earlier than that in the controls (at week 78), the eosinophilic pre-

neoplastic lesions could be induced by DDT as a result of DNA damage of hepatocytes exposed to oxidative stress as described before. It is conceivable that DDT may acceler-

Table 15. Mean area of altered hepatocellular foci (AHF) in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	No. of rats examined		Mean area of AHF (mm ²): Mean \pm SD (No. of rats with AHF)					
				Eosinophilic		Tigroid basophilic		Clear cell	
		Male	Female	Male	Female	Male	Female	Male	Female
0	26	6	6	-	-	0.02 \pm 0.004 (2)	0.03 \pm 0.004 (2)	-	-
	52	6	6	-	-	0.06 \pm 0.05 (3)	0.13 \pm 0.09 (4)	0.02 \pm 0.02 (5)	-
	78	8	8	0.16 (1)	-	0.09 \pm 0.03 (8)	0.11 \pm 0.03 (8)	0.05 \pm 0.02 (5)	0.04 \pm 0.01 (3)
	104	10	10	0.33 \pm 0.16 (8)	0.46 \pm 0.18 (3)	0.16 \pm 0.04 (9)	0.17 \pm 0.08 (10)	0.08 \pm 0.04 (9)	0.09 \pm 0.08 (5)
5	26	6	6	-	-	0.03 (1)	0.02 (1)	0.03 (1)	-
	52	6	6	-	-	0.03 (1)	0.09 \pm 0.03 (6)	0.03 \pm 0.01 (4)	-
	78	8	8	0.60 \pm 0.63 (2)	0.04 (1)	0.09 \pm 0.05 (8)	0.08 \pm 0.03 (8)*	0.05 \pm 0.03 (6)	0.05 \pm 0.02 (2)
	104	10	10	0.44 \pm 0.57 (7)	0.38 \pm 0.39 (4)	0.11 \pm 0.04 (9)**	0.14 \pm 0.03 (10)	0.08 \pm 0.04 (10)	-
50	26	6	6	-	-	0.06 (1)	0.03 \pm 0.003 (3)	0.03 (1)	-
	52	6	6	-	-	-	0.09 \pm 0.02 (6)	0.05 \pm 0.02 (4)	-
	78	8	7	0.21 \pm 0.17 (8)	0.32 (1)	0.25 \pm 0.49 (8)	0.10 \pm 0.04 (7)	0.09 \pm 0.06 (8)	-
	104	10	10	0.58 \pm 0.54 (9)	0.35 \pm 0.21 (2)	0.11 \pm 0.06 (9)*	0.15 \pm 0.03 (10)	0.18 \pm 0.24 (10)	0.05 \pm 0.03 (2)
500	26	6	6	0.05 \pm 0.03 (4)	-	-	0.07 \pm 0.06 (2)	-	-
	52	6	6	0.06 \pm 0.03 (6)	0.06 \pm 0.02 (2)	-	-	-	-
	78	7	8	0.32 \pm 0.18 (7)	0.26 \pm 0.13 (8)	0.07 \pm 0.02 (3)	0.09 \pm 0.06 (6)	-	-
	104	10	10	0.90 \pm 0.43 (10)**	1.02 \pm 0.70 (10)	0.17 \pm 0.10 (10)	0.08 \pm 0.02 (8)**	0.41 \pm 0.53 (4)	0.03 \pm 0.01 (4)

-: Not available because of no AHF.

** *: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Fisher's exact test).

Table 16. Area fraction of liver occupied by altered hepatocellular foci (AHF) in F344 rats from a 2-year feeding study of *p,p'*-DDT

Dose (ppm)	Duration (weeks)	No. of rats examined		Area fraction occupied by AHF (%): Mean \pm SD					
				Eosinophilic		Tigroid basophilic		Clear cell	
		Male	Female	Male	Female	Male	Female	Male	Female
0	26	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.01	0.02 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00
	52	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.08 \pm 0.16	0.26 \pm 0.42	0.04 \pm 0.04	0.00 \pm 0.00
	78	8	8	0.02 \pm 0.05	0.00 \pm 0.00	0.18 \pm 0.10	1.06 \pm 0.54	0.04 \pm 0.05	0.02 \pm 0.03
	104	10	10	0.51 \pm 0.41	0.25 \pm 0.44	0.98 \pm 0.70	1.82 \pm 1.16	0.16 \pm 0.18	0.08 \pm 0.13
5	26	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.005 \pm 0.012	0.01 \pm 0.01	0.01 \pm 0.01	0.00 \pm 0.00
	52	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.02	0.41 \pm 0.26	0.04 \pm 0.04	0.00 \pm 0.00
	78	8	8	0.31 \pm 0.77	0.01 \pm 0.02	0.21 \pm 0.11	0.85 \pm 0.52	0.06 \pm 0.07	0.02 \pm 0.04
	104	10	10	1.07 \pm 1.85	0.20 \pm 0.38	0.54 \pm 0.42	1.93 \pm 0.45	0.19 \pm 0.16	0.00 \pm 0.00*
50	26	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.05	0.03 \pm 0.03	0.005 \pm 0.011	0.00 \pm 0.00
	52	6	6	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.47 \pm 0.12	0.06 \pm 0.09	0.00 \pm 0.00
	78	8	7	0.60 \pm 0.39*	0.18 \pm 0.48	0.34 \pm 0.48	0.89 \pm 0.53	0.12 \pm 0.08	0.00 \pm 0.00
	104	10	10	5.10 \pm 4.74*	0.32 \pm 0.71	0.41 \pm 0.32	2.54 \pm 0.76	0.42 \pm 0.52	0.02 \pm 0.04
500	26	6	6	0.04 \pm 0.05**	0.00 \pm 0.00	0.00 \pm 0.00	0.03 \pm 0.07	0.00 \pm 0.00	0.00 \pm 0.00
	52	6	6	0.23 \pm 0.09**	0.08 \pm 0.14	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	78	7	8	2.72 \pm 1.05**	3.01 \pm 1.84**	0.03 \pm 0.05*	0.10 \pm 0.10**	0.00 \pm 0.00	0.00 \pm 0.00
	104	10	10	9.25 \pm 6.28**	8.25 \pm 4.53**	0.31 \pm 0.19	0.10 \pm 0.09**	0.14 \pm 0.30	0.03 \pm 0.03

** *: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Fisher's exact test).

ate the occurrence of initiated cells by oxidative stress, leading to the early appearance of eosinophilic AHF and promotes the growth of eosinophilic preneoplastic lesions through its mitogenic activity in combination with the inhibitory effect on GJIC as mentioned before. In other types of AHF, tigroid basophilic AHF decreased in number

and size in females in the high dose group. A similar result was also reported in rats treated with phenobarbital (33).

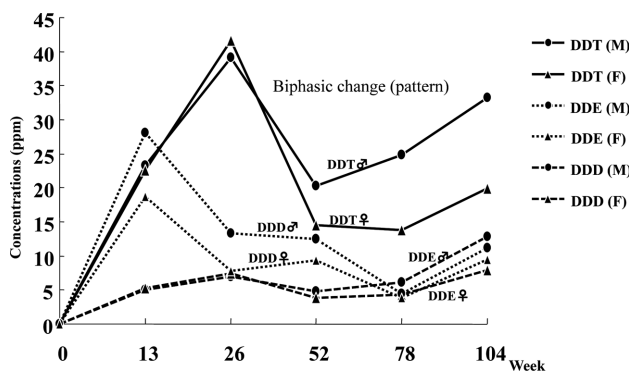
As to neoplasia, the overall incidences of hepatocellular adenomas and carcinomas in the high-dose group during the study were 55% and 35% for males and 40% and 5% for females, respectively (Table 17). Morphologically, the hepa-

Table 17. Incidence of hepatocellular tumors in F344 rats from a 2-year feeding study of *p,p'*-DDT

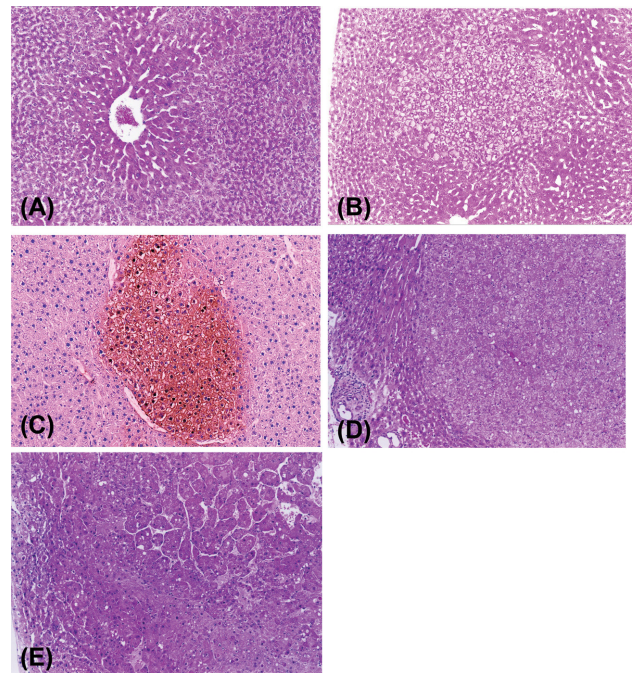
Dose (ppm)	Duration (weeks)	Adenoma		Carcinoma	
		Male	Female	Male	Female
0	26	0/6	0/6	0/6	0/6
	52	0/6	0/6	0/6	0/6
	78	0/8	0/8	0/8	0/8
	104	0/35	0/33	0/35	0/33
	Total ^a	0/40	0/40	0/40	0/40
5	26	0/6	0/6	0/6	0/6
	52	0/6	0/6	0/6	0/6
	78	0/8	0/8	0/8	0/8
	104	0/30	0/27	0/30	0/27
	Total	0/40	0/40	0/40	0/40
50	26	0/6	0/6	0/6	0/6
	52	0/6	0/6	0/6	0/6
	78	0/8	0/7	0/8	0/7
	104	5/36*	0/32	0/36	0/32
	Total	5/40*	0/40	0/40	0/40
500	26	0/6	0/6	0/6	0/6
	52	0/6	0/6	0/6	0/6
	78	6/7**	1/8	0/7	0/8
	104	20/33**	16/33**	13/33**	2/33
	Total	22/40**	16/40**	14/40**	2/40

^a: Total number of animals examined (No. of scheduled deaths after 104 weeks + No. of unscheduled deaths).

*, **: Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively (Fisher's exact test).

**Fig. 3.** Liver concentrations of DDT, DDE, and DDD in F344 rats at high-dose (500 ppm) in a 2-year feeding study. (M) male; (F) female.

hepatocellular adenomas contained typically eosinophilic hepatocytes (Fig. 4D) which were similar to those of eosinophilic AHF described above and often had basophilic phenotypes in small numbers. Basophilic cell type of adenomas was also noted but only in a few animals. On the other hand, the hepatocellular carcinomas contained hepatocytes with eosinophilic or basophilic cytoplasm and typically had admixture of both phenotypes (Fig. 4E). The population of basophilic

**Fig. 4.** Centrilobular hepatocellular hypertrophy in a male F344 rat treated with DDT at 500 ppm for 26 weeks (A), H&E stain, $\times 112$; An eosinophilic focus with ground glass appearance in a male F344 rat treated with DDT at 500 ppm for 78 weeks (B), H&E stain, $\times 54$; GST-P positive focus in a male F344 rat treated with DDT at 500 ppm for 52 weeks, which was eosinophilic type of focus in H&E-stained section (C), GST-P stain, $\times 112$; Hepatocellular adenoma comprising eosinophilic hepatocytes with pale pink or ground glass appearance cytoplasm in a male F344 rat treated with DDT at 500 ppm for 104 weeks (D), H&E stain, $\times 85$; Hepatocellular carcinoma containing both eosinophilic and basophilic phenotypes of hepatocytes with trabecular pattern in a male F344 rat treated with DDT at 500 ppm for 104 weeks (E), H&E stain, $\times 85$.

cells in the carcinomas was much higher than that in adenomas or large eosinophilic AHF. Since the hepatocytes within the majority of adenomas were morphologically similar to those of eosinophilic AHF and there was no increase in other types of AHF, it was suggested that the eosinophilic AHF could develop into neoplasms without passing an intermediate stage (37). However, large eosinophilic AHF and adenomas sometimes contained small number of basophilic cells within the lesions. The presence of basophilic cells might be an indication of malignant transformation of hepatocytes within the lesions since the population of basophilic phenotypes is largest in the hepatocellular carcinomas (37). A similar type of nongenotoxic hepatocarcinogen, phenobarbital (PB), also has been shown to induce eosinophilic altered foci and hepatocellular tumors containing eosinophilic cells (GGT positive) (33). However, it should be recognized that the occurrence of eosinophilic AHF is not limited to PB and DDT because many other chemicals

Table 18. Microarray assay of hepatoproliferative lesions in male F344 rats from a 2-year feeding study of *p,p'*-DDT

Gene/protein name	Function	Fold change (ratio to normal control value)*			
		Hypertrophy		Eosinophilic AHF	
		500 ppm	0 ppm	500 ppm	500 ppm
Protein kinase C-eta	Cell proliferation (CP)	3.6	3.2	3.1	2.5
Uncouplingprotein 2, mitochondrial	CP	2.0	-	2.0	-
Phosphorylase B kinase catalytic subunit	CP	-	-	2.2	-
Guanine nucleotide-binding protein G-s, α subunit	CP	2.5	-	2.6	2.3
G protein γ -5 subunit	CP	2.0	-	3.5	-
CDK106	CP (Downreg. TGF- β)	-	-	2.0	2.0
Follistatin	CP (Downreg. TGF- β)	-	-	0.5	-
Lipoprotein lipase	CP (TGF- β)	-	-	0.5	-
Ubiquitin-conjugating enzyme E2D3	CP (Cell cycle)	-	-	0.5	-
p53-activated gene 608	Apoptosis (Apo)	-	-	2.3	2.0
Sodium channel β 2	Apo (suppre)	2.9	2.2	3.2	2.4
Protein disulfide isomerase A6 precursor	Apo (suppre)	-	-	0.5	-
Protein phosphatase type 1 α , catalytic subunit	Apo (suppre)	0.5	-	0.4	-
Potassium channel gene 1 (Kir6)	Anti-oxidative	3.2	2.7	3.1	2.5
Dopamine receptor D2	Anti-oxidative	2.7	3.0	2.1	2.2

*: Values of 2.0 or more and 0.5 or less represent up-regulation and down-regulation, respectively.

–: Within normal range.

including genotoxic agents also induced such eosinophilic preneoplastic lesions (37,38). In addition, it has been suggested that the development of hepatocellular tumors in rats and mice after long-term exposure to CAR activators such as PB and DDT seems to be rodent-specific and not relevant to human (24).

With respect to sex difference in tumor development, it is generally believed that males have a higher incidence of hepatic tumors than females in rodents as well as in humans (39). Factors contributing to this sex difference have not been clearly demonstrated, but it may be due to the difference of hormonal pattern which is of primary importance for determining the metabolic activation of carcinogens and also due to the sex chromosome in its role as a carrier of genetic messages (39). In our 2-year rat study, the incidence of hepatocellular tumors, especially carcinomas, was much higher in males than females. This sex difference might be partly due to the higher concentrations of DDT and its metabolites (DDE and DDD) in the liver of males (Fig. 3) which may result in higher production of oxidative stress through metabolic activation. In fact, the levels of LPO and 8-OHdG were much higher in males than females. In addition, the higher incidence of spontaneous eosinophilic foci in male F344 rats (40) may contribute to the sex difference. In other words, the male liver may have a preferable microenvironment for occurrence of eosinophilic AHF. The reduced body weight gain in the high-dose group which was more evident in females than males (5) was also considered as an influential factor for tumor development, but it seems to have no correlation with the occurrence of hepatocellular tumors in F344 rats (41).

Gene expression in target sites. Analysis of gene expression by microarray following laser-captured microdissection was performed on the target tissues including hepatocellular hypertrophy, eosinophilic AHF, and hepatocellular adenoma observed in the high-dose and/or control rats. The results are summarized in Table 18. As shown in Table 18, various genes relating to cell proliferation, apoptosis or anti-oxidative function were up-regulated or down-regulated in the target sites and the most prominent changes were noted in the eosinophilic AHF from the high-dose rat. These gene expressions might be corresponding changes to the mitogenic activity of DDT and/or oxidative stress generated through the metabolic activation. However, we need further accumulation of gene expression data to arrive at conclusion since the information from our results is quite limited.

CONCLUSIONS

DDT and its metabolites may have an endocrine disrupting potential affecting reproductive system although the effects may vary among animal species in correlation with exposure levels. Epidemiologic studies revealed either positive or negative associations between exposure to DDT and tumor development, but there has been no clear evidence that DDT causes cancer in humans. In experimental animals, tumor induction by DDT has been shown in the liver, lung, and adrenal. The mechanisms of hepatic tumor development by DDT has been studied in rats and mice. DDT is known as a non-genotoxic hepatocarcinogen and has been shown to induce microsomal enzymes through activation of

constitutive androstane receptor (CAR) and to inhibit gap junctional intercellular communication (GJIC) in the rodent liver. The results from our previously conducted 4-week and 2-year feeding studies of *p,p'*-DDT in F344 rats indicate that DDT may induce hepatocellular eosinophilic foci as a result of oxidative DNA damage and leads them to hepatic neoplasia in combination with its mitogenic activity and inhibitory effect on GJIC. Oxidative stress could be a key factor in hepatocarcinogenesis by DDT.

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