Review

Hepatocarcinogenesis by DDT in Rats

Takanori Harada¹, Ryoichi Ohtsuka¹, Makio Takeda¹, Toshinori Yoshida¹,
Akiko Enomoto¹, Sayuri Kojima¹, Naruto Tomiyama¹,
Nobuaki Nakashima¹, and Masakazu Ozaki²

¹The Institute of Environmental Toxicology, 4321 Uchimoriya-machi, Joso-shi, Ibaraki 303–0043, Japan

²Sumika Technoservice Corporation, 1–98 Kasugadenaka, 3-chome Konohana-ku, Osaka 554–8558, Japan

Abstract: DDT is known as a nongenotoxic hepatocarcinogen and has been shown to induce microsomal enzymes and GGT-positive foci and to inhibit gap junctional intercellular communication (GJIC) in the rodent liver. In consideration of these findings, we examined time-related changes in potential factors including hepatic enzyme induction, oxidative stress, cell proliferation, and GJIC in a 4-week and a 2-year feeding studies of *p,p'*-DDT with F344 rats. Consequently, hepatic microsomal P450 isozymes such as CYP2B1 and CYP3A2 were induced from the beginning of treatment in a dose-dependent manner. Measurement of oxidative stress markers revealed significant increases in lipid peroxide and 8-hydroxydeoxyguanosine at dose levels that induced hepatocellular tumors. Cell proliferation was enhanced within 3 days at any dose level, but returned to normal after 7 days and no significant changes thereafter. GJIC was inhibited throughout the study from the beginning to the end of treatment. Histologically, eosinophilic foci appeared earlier than controls and increased in number and size prior to development of hepatocellular tumors. These results indicate that DDT may induce eosinophilic foci as a result of oxidative DNA damage and leads them to neoplasia in combination with its mitogenic activity and inhibitory effect on GJIC. Oxidative stress could be a key factor in hepatocarcinogenesis by DDT. (*J Toxicol Pathol* 2006; **19**: 155–167)

Key words: enzyme induction, oxidative stress, cell proliferation, intercellular communication, eosinophilic foci, DDT

Introduction

DDT is still used in the tropics and subtropics for the control of malaria and other insect-transmitted diseases, although the use of this compound has been banned or restricted to certain areas in many countries since 1970s because of its chemical characteristics such as accumulation and bioconcentration in lipid systems of all animal species which may result in occurrence of potential adverse effects on humans and wild animals. DDT and its metabolites have been extensively studied for their toxicity and carcinogenicity in experimental animals and shown to have a carcinogenic effect on the liver¹. DDT is classified as a nongenotoxic hepatocarcinogen based on the results of genotoxicity studies¹ and has been shown to induce microsomal enzymes^{2–5} and GGT-positive foci² and to inhibit gap junctional intercellular communication (GJIC)^{6–10} in the rodent liver. In consideration of these findings, we have conducted further mechanistic studies to

examine time-related changes in cell proliferation, GJIC, enzyme induction, oxidative stress and histological alterations including altered hepatocellular foci (AHF) during the carcinogenic process in the liver of F344 rats treated with *p,p'*-DDT¹¹. This paper describes the overview of the time-related changes in these parameters based on our results¹¹, and potential factors which might be involved in the hepatocarcinogenesis by DDT are discussed.

Hepatic Microsomal Enzyme Induction

Our analyses of hepatic microsomal enzymes revealed significant increases in pentoxeresorufin 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^{2,7,8,12}. 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

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Mailing address: Takanori Harada, The Institute of Environmental Toxicology, 4321 Uchimoriya-machi, Joso-shi, Ibaraki 303–0043, Japan

TEL: 81-297-27-4520 FAX: 81-297-27-4518

E-mail: harada@iet.or.jp

concurrent incidence of preneoplastic eosinophilic foci and hepatocellular tumors. As for 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)⁵. Since CYP3A2 is androgen-dependent and not normally expressed in adult female rats¹³, 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 metabolism⁵. The preferential induction of CYP2B1 and CYP3A2 by DDT in female rats indicates an endocrine disrupting 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 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^{12,14}. Since 8-OHdG levels were significantly increased in the DDT-treated groups that developed hepatocellular carcinomas, 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 mispairing (mutation) on DNA replication¹⁵.

In our 2-year 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 nongenotoxic mitogenic hepatocarcinogens^{16–18}. It is generally known that the hepatic cell proliferation response to nongenotoxic mitogenic hepatocarcinogens such as phenobarbital typically occurs through an initial burst of enhanced DNA synthesis followed by enhanced mitosis^{17–19}. 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 treatment¹⁸. It has been postulated that nongenotoxic chemicals with mitogenic activity may provide a selective growth advantage to spontaneously initiated precancerous cells over normal hepatocytes and lead them to neoplasms^{16,17}. 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 nongenotoxic tumor-promoting agents including phenobarbital and DDT *in vivo* and *in vitro*^{2,6–8,10,20–22}. The inhibition of GJIC by tumor promoters may be produced in several ways⁹. 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^{7,22,23}. Therefore, inhibition of GJIC may isolate initiated cells from the growth regulatory signals of neighboring cells and permit the clonal expansion of initiated 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 proliferation¹⁶. Upon

Fig. 1. Centrilobular hepatocellular hypertrophy in a male F344 rat treated with DDT at 500 ppm for 26 weeks. H&E, $\times 112$.

Fig. 2. A small eosinophilic focus in a male F344 rat treated with DDT at 500 ppm for 26 weeks. H&E, $\times 170$.

continued administration, the increased liver weight is maintained, even though the rate of cell turnover returns to normal levels¹⁶. 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 study, treatment with DDT induced centrilobular hepatocellular hypertrophy and increased eosinophilic AHF, hepatocellular adenomas and carcinomas (Table 1–3). The severity of hepatocellular hypertrophy (Fig. 1) 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. 2) and sometimes had cytoplasmic clear spaces which may represent glycogen deposits. The eosinophilic AHF was positive for GST-P (Fig. 3). 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

Fig. 3. 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. GST-P stain, $\times 112$.

Table 1. Time-Related Changes in Hepatic Microsomal P450 Isozyme Content in Male F344 Rats from a 4-Week Feeding Study of 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. Time-Related Changes in Hepatic Microsomal Enzyme Activity and P450 Isozyme Contents in Male F344 Rats from a 2-Year Feeding Study of 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. Time-Related Changes in Hepatic Microsomal Enzyme Activity and P450 Isozyme Contents in Female F344 Rats from a 2-Year Feeding Study of 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).

Fig. 4. A large eosinophilic focus in a male F344 rat treated with DDT at 500 ppm for 78 weeks. A small number of basophilic cells are also seen within the lesion. H&E, $\times 54$.

Fig. 5. 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. H&E, $\times 85$.

perivenular hepatocellular hypertrophy, being closely related to the later development of preneoplastic hepatic foci including clear/acidophilic foci as well as hepatocellular neoplasms²⁴.

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% 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 preneoplastic 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 accelerate 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 phenobarbital²¹.

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

Fig. 6. 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. H&E, $\times 85$.

Table 4. Time-Related Changes in Hepatic Lipid Peroxide (LPO) Contents in F344 Rats from a 2-Year Feeding Study of 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. Time-Related Changes in Hepatic 8-OHdG Levels in F344 Rats from a 2-Year Feeding Study of DDT

Dose (ppm)	Sex	No. of rats examined	8-OHdG (ng/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).

Table 6. Time-Related Changes in PCNA LI (%) in the Liver of Male F344 Rats from a 4-Week Feeding Study of DDT

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

(n): Number of animals examined.

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

females, respectively (Table 17). Morphologically, the hepatocellular adenomas contained typically eosinophilic hepatocytes (Fig. 5) 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. 6). The

population of basophilic 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 stage²⁵. However, large eosinophilic AHF and adenomas sometimes contained small number of basophilic cells within the

Table 7. Time-Related Changes in PCNA LI (%) in the Liver of F344 Rats from a 2-Year Feeding Study of DDT

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

(n): Number of animals examined.

Table 8. Time-Related Changes in the Number of GJIC Protein Cx32 Spots in the Liver of Male F344 Rats from a 4-Week Feeding Study of DDT

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

(n): Number of animals examined.

: Significantly different from control at $p < 0.01$ (Dunnett's multiple comparison test).Table 9.** Time-Related Changes in the Number of GJIC Protein Cx32 Spots in the Liver of F344 Rats from a 2-Year Feeding Study of DDT

Dose (ppm)	Sex	No. of Cx32 spots (mean $\pm$ SD) at weeks			
		26	52	78	104
0	M	7.07 $\pm$ 0.94 (6)	6.49 $\pm$ 1.46 (6)	3.05 $\pm$ 0.19 (6)	3.11 $\pm$ 1.15 (5)
	F	6.08 $\pm$ 0.97 (6)	6.48 $\pm$ 0.66 (6)	3.26 $\pm$ 0.74 (6)	3.73 $\pm$ 0.23 (5)
5	M	6.87 $\pm$ 0.54 (6)	6.95 $\pm$ 0.88 (6)	2.51 $\pm$ 0.50* (6)	2.13 $\pm$ 0.37 (5)
	F	5.67 $\pm$ 0.93 (6)	5.51 $\pm$ 1.62 (6)	2.90 $\pm$ 0.41 (6)	3.59 $\pm$ 0.42 (6)
50	M	5.53 $\pm$ 0.83* (6)	6.28 $\pm$ 1.88 (6)	2.47 $\pm$ 0.44* (6)	2.13 $\pm$ 0.35 (4)
	F	4.80 $\pm$ 0.86 (6)	4.20 $\pm$ 0.56** (6)	1.76 $\pm$ 0.69* (6)	2.65 $\pm$ 0.33** (6)
500	M	5.39 $\pm$ 1.30* (6)	4.64 $\pm$ 0.91 (6)	1.14 $\pm$ 0.22** (6)	1.52 $\pm$ 0.28* (6)
	F	4.20 $\pm$ 1.41* (6)	3.28 $\pm$ 0.72** (6)	1.03 $\pm$ 0.16** (6)	1.75 $\pm$ 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. Time-Related Changes in Liver Weights of Male F344 Rats from a 4-Week Feeding Study of 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. Time-Related Changes in Liver Weights of Male F344 Rats from a 2-Year Feeding Study of 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. Time-Related Changes in Liver Weights of Female F344 Rats from a 2-Year Feeding Study of 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).

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 carcinomas²⁵. 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)²¹. However, it should be recognized that the occurrence of eosinophilic AHF is not limited to PB and DDT because many other chemicals including genotoxic

agents also induced such eosinophilic preneoplastic lesions^{25,26}.

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 humans²⁷. 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

Table 13. Incidences of Hepatocellular Hypertrophy and Altered Hepatocellular Foci (AHF) in F344 Rats from a 2-Year Feeding Study of 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 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).

a carrier of genetic messages²⁷. In our 2-year 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 males¹¹ 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 rats²⁸ may contribute to the sex difference. In other words, the male liver may have a preferable microenvironment for occurrence of eosinophilic AHF. The

Table 15. Mean Area of Altered Hepatocellular Foci (AHF) in F344 Rats from a 2-Year Feeding Study of 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 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).

reduced body weight gain in the high-dose group which was more evident in females than males¹¹ 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 rats²⁹.

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 upregulated or downregulated 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

Table 17. Incidence of Hepatocellular Tumors in F344 Rats from a 2-Year Feeding Study of 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).

Table 18. Microarray Assay of Hepatoproliferative Lesions in Male F344 Rats from a 2-Year Feeding Study of DDT

Gene / protein name	Function	Fold change (ratio to normal control value)*			
		Hypertrophy	Eosinophilic AHF		Adenoma
		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 upregulation and downregulation, respectively.

–: Within normal range

activation. However, we need further accumulation of gene expression data to arrive at conclusion since the information from our results is quite limited.

Conclusion

Our study results indicate that DDT may induce eosinophilic AHF as a result of oxidative DNA damage of hepatocytes and promote the progression of the preneoplastic lesions into hepatocellular tumors in

combination with its mitogenic activity and inhibitory effect on GJIC. The production of oxidative stress (increases in LPO and 8-OHdG) which might be secondary to metabolic activation could be a key factor in hepatocarcinogenesis by DDT and may play an important role in tumor promotion and progression (malignant transformation).

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