Effects of a Polychlorinated Biphenyl Mixture (Aroclor 1254) and DDT on Biliary Thyroxine Excretion in Rats[1]

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ABSTRACT. Treatment of rats with a polychlorinated biphenyl mixture (Aroclor 1254; PCB) caused a 4- to 5-fold increase in the 3-hr biliary excretion of ^{125}I-thyroxine (T_4). In treated rats bile flow was increased and the ^{125}I-T_4 bile:plasma ratios (B/P ratios) and biliary clearance rate of plasma ^{125}I-T_4 were greatly elevated. The proportion of biliary ^{125}I present as T_4-glucuronide was larger in PCB-treated rats. PCB also elevated thyroid ^{131}I uptake and reduced serum PBI concentrations.

DDT did not affect the biliary excretion of ^{125}I-T_4, but did increase bile flow and the biliary clearance rate of plasma ^{125}I-T_4. It also caused a marginal elevation of B/P ratios and increased the proportion of biliary ^{125}I present as T_4-glucuronide slightly but significantly. DDT did not affect thyroid ^{131}I uptake or serum PBI concentrations.

The effects of PCB were similar to those of the polycyclic hydrocarbon-type of enzyme inducers. The results of DDT-treatment were similar to those produced by the phenobarbital type of inducer. PCB treatment also reduced the binding of thyroid hormone to serum proteins as shown by enhanced resin uptake of ^{125}I-triiodothyronine (T_3) from the serum of PCB-treated rats; DDT administration did not influence resin T_3 uptake. (*Endocrinology* **95**: 1150, 1974)

THE most widespread environmental pollutants are first, chlorinated hydrocarbon pesticides, such as DDT (1,2) and secondly, polychlorinated biphenyls (PCB's) (3–5). DDT is still used by many American farmers to control pests attacking a variety of crops (6). PCB's have many industrial applications, including use in dielectric fluids, in heat transfer and hydraulic fluids, in gas turbine engines and vaccum pumps, in paints and varnishes and as plasticizers, adhesives and textile coatings (7). Like DDT, PCB residues have been detected in rivers, oceans, fish, wildlife, cattle, poultry and in human hair, milk, adipose tissue, serum and excreta (3–5,8–10). DDT and PCB's are structurally similar; they are stable and persist for long periods in the environment and, being fat soluble, accumulate up the food chain (3,5).

DDT (11,12) and PCB's (13) produced goiters in birds and treatment of rats with DDD isomers increased thyroid weight and fecal thyroxine (T_4) excretion (14). Another chlorinated hydrocarbon pesticide, chlordane, enhanced thyroid function (15) and augmented hepatic accumulation of ^{125}I-T_4 in rats (16). Both DDT and PCB's are hepatic microsomal enzyme inducers (17–20). Since other enzyme inducers, such as polycyclic hydrocarbons (21–23) and phenobarbital (15,21), enhance biliary T_4 excretion, the effects of DDT and a PCB mixture on biliary T_4 excretion were studied.

Materials and Methods

Male hooded rats, weighing 280–300 g, were obtained from the Canadian Breeding Laboratories, St.-Constant, Quebec and were maintained on Purina Laboratory Chow. The Industrial Nuclear Co., St. Louis, Mo., supplied L-^{125}I-T_4; the specific activity when used was 50–100 mCi/mg and 92–95% of the ^{125}I was in the form of T_4 by paper chromatography. The PCB mixture (Aroclor 1254) was a gift from the Monsanto Chemical Co., St. Louis, Mo. The Aroclors are designated by four digits; the first two represent the molecular type (12-chlorinated biphenyls) and the last two give the weight percent of chlorine (54–54% chlorine). The Nutritional Biochemical Co., Cleveland, Ohio, supplied DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl) ethane]. Both compounds

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were dissolved in corn oil to give concentrations of 15 mg/ml. Treated rats received 25 mg (70.5 μmoles of DDT or 77.2 μmoles of PCB) per kg ip daily for 4 days; control animals received corn oil alone.

Biliary excretion of ^{125}I-T_4. Twenty-four hr after the last injection, under pentobarbital anesthesia, the bile ducts of the rats were cannulated as previously described (23,24). When bile was flowing freely, 0.1μg ^{125}I-T_4 in 0.5 ml 0.9% NaCl was injected into the right jugular vein. Bile was collected in 20-min periods for 3 hr. Plasma was obtained at the mid-point of each period (24). Bile volumes (ml/hr/kg), bile:plasma ^{125}I ratios (B/P ratios) and biliary clearance (B/P ratio × bile flow) of plasma ^{125}I-T_4 (ml/hr/kg) were calculated as previously outlined (23,24). From the radioactivity in an aliquot of the administered dose, the excretion of ^{125}I-T_4 was calculated. Aliquots of the first and then alternate bile samples were chromatographed on paper in a 1-butanol:1,4-dioxan:NH$_4$OH system (23,24), radioautographs were prepared and the proportion of biliary radioactivity present as T_4-glucuronide, T_4 and I^- was calculated as previously detailed (23,24).

Thyroid ^{131}I *uptake and serum PBI.* Eight hr after the last injection of DDT, PCB or corn oil, rats were given 0.5 μCi of carrier-free ^{131}I in 0.2 ml 0.9% NaCl ip. Seventeen hr later, under ether anesthesia, blood was withdrawn from the abdominal aorta and the thyroid gland attached to a piece of trachea was removed. Serum (1.5 ml) was passed through 2 × 1 cm columns of AmberlIte IRA-400 ion exchange resin (Mallinckrodt Chemical Works, St. Louis, Mo.) to remove I^- before determining the serum PBI by AutoAnalyzer (25).

^{125}I-T_3 *resin uptake from serum.* Twenty-four hr after the last injection of corn oil, PCB or DDT, rats were exsanguinated from the abdominal aorta under ether anesthesia. A fourth group of animals were fed Purina chow containing 0.1% propylthiouracil (PTU) for 80 days. A Res-O-Mat ^{125}I-T_3 Diagnostic Kit was obtained from the Mallinckrodt Chemical Works, St. Louis, Mo. The test was performed as recommended by the manufacturer (26), except that 1.0 ml instead of 0.5 ml of serum was used. The control human serum and "normalizing factor" were not used. From the radioactivity remaining in the vials after the 2-hr mixing period, the percent ^{125}I-T_3 taken up by the resin was calculated. In another experiment serum from 6 untreated rats was pooled. To 7 ml of the pool 6 mg PCB was added; the serum was centrifuged and the undissolved PCB settled in a bead at the bottom of the tube. The T_3 resin uptake was determined in the untreated and PCB-treated serum.

Results

Figure 1 shows the cumulative biliary excretion of ^{125}I-T_4. In PCB-treated rats

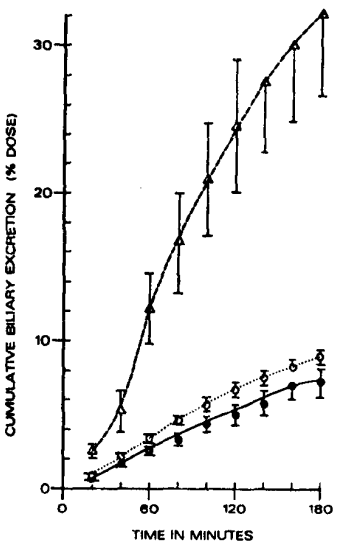

FIG. 1. Cumulative biliary excretion of ^{125}I-T_4 in control (● — ●), DDT- (○ ··· ○) and PCB- (Δ – – – Δ) treated rats; the same symbols are used in Figs. 2–5. Each value is the mean of 4 determinations; brackets indicate SEM. In all figures the statistical significance of the difference between means was determined by Student's *t* test. Biliary excretion in the PCB group was significantly different from controls at all time periods ($p < 0.001$); in the DDT group no value was significantly different from control.

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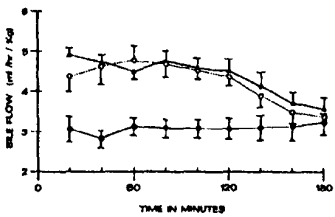

FIG. 2. Bile flow (ml/hr/kg); each point is the mean ± SEM of 4 determinations. Values in PCB-treated rats were significantly higher than in controls ($p < 0.01$), except in the last 2 periods; values in DDT-treated rats were also significantly higher ($p < 0.05 - < 0.01$), except in the last 3 periods.

biliary T_4 excretion was increased 4- to 5-fold at all time intervals; at 20 min, control animals excreted 0.6% of the dose compared to 2.5% in treated rats and at 3 hr the figures were 7 and 32%. In DDT-treated rats excretion was slightly higher than in controls, but at no point did it reach significance; at the end of the 3 hr, treated rats excreted 9% of the dose compared to 7% in controls.

Bile flow is illustrated in Fig. 2. Both DDT and PCB significantly enhanced bile flow, except towards the end of the collection periods. In control animals bile flow was approximately 3.0 ml/hr/kg; flow in the treated rats (DDT and PCB) was about 4.5 ml/hr/kg.

B/P ratios are shown in Fig. 3. In control and DDT-treated rats the B/P ratio at 20 min was 0.5 and then rose to and remained at 1.0. The ratios were similar in both groups except at 120 and 180 min, when the B/P ratios in DDT-treated rats were slightly, but significantly, elevated. In contrast the B/P ratios in PCB-treated rats were markedly elevated at all time intervals. At 20 min it was 4.5, rose to 11 at 40 min and then remained at a mean of 12.5 to 14.0; between 1 and 3 hr the lowest individual value was 7.0 and the highest 26.0.

Figure 4 depicts the biliary clearance rate of circulating ^{125}I-T_4 (B/P ratio × bile flow). In control rats the clearance rate at 20 min was 1.5 ml/hr/kg and then rose to and remained at approximately 3.0–3.5 ml/hr/kg. In DDT-treated rats the biliary clearance rate after the first 20 min was 4.5–5.5 ml/hr/kg, significantly higher than in controls. In PCB-treated rats the clear-

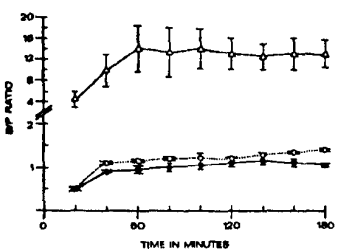

FIG. 3. B/P ratios; each point is the mean ± SEM of 4 determinations. Values in PCB-treated rats were significantly higher than in controls ($p < 0.001$) at all time periods; none of the values in DDT-treated rats was significantly different from controls, except in the last 2 periods ($p < 0.02 - < 0.01$).

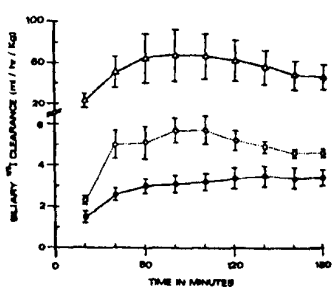

FIG. 4. Biliary clearance rate (ml/hr/kg) of plasma ^{125}I-T_4; each point is the mean ± SEM of 4 determinations. Values in PCB-treated rats were significantly higher than in controls ($p < 0.001$) at all time periods; values in DDT-treated rats were also significantly increased ($p < 0.05 - < 0.02$), except in the 1st and 8th periods.

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ance rate was greatly increased; 23 ml/hr/kg at 20 min and then 50–70 ml/hr/kg. Between 1 and 3 hr the lowest individual value was 31 and the highest was 138 ml/hr/kg.

In control animals 1 ml plasma contained 4.6% of the administered ^{125}I at 10 min (Fig. 5), falling to 3.7% at 30 min and then gradually declining to 2.2% at 170 min. In DDT-treated animals these values were significantly lower, namely 3.7, 2.5 and 1.5%. In PCB-treated rats the values were still lower at 1.3, 1.0 and 0.5%.

Figure 6 shows the percent of total biliary radioactivity present as T_4-glucuronide, T_4 and I^- in the first and then alternate bile samples. In control animals 20% of biliary ^{125}I was in T_4-glucuronide in the 0–20-min sample; the proportion then rose to about 50%. In PCB-treated rats 65% of biliary ^{125}I was in T_4-glucuronide in the 0–20-min sample and thereafter was almost 80%. In DDT animals the values were 29 and 52–57%, slightly but significantly higher than in controls, except in the last 2 collection periods. The proportion of biliary radioactivity present as free T_4 was reduced in DDT- and PCB-treated rats, particularly in the latter. The proportion of ^{125}I present as I^- was unchanged in DDT-

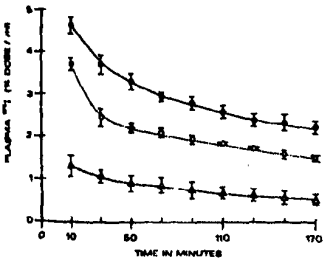

FIG. 5. Plasma concentration (% dose/ml) of ^{125}I-T_4; each point is the mean ± SEM of 4 determinations. All values were lower than control in PCB- ($p < 0.001$) and DDT- ($p < 0.01 - < 0.001$) treated rats.

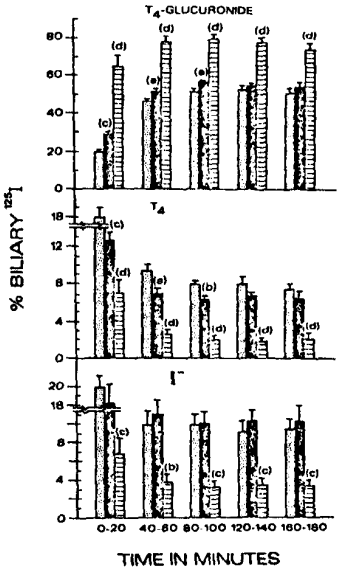

FIG. 6. Percent distribution of total biliary ^{125}I in T_4-glucuronide, free T_4 and I^-; mean ± SEM of 4 determinations. Stippled bars—controls; black bars—DDT; cross-lined bars—PCB. P (vs control): a < 0.05; b < 0.02; c < 0.01; d < 0.001.

treated compared to control rats, but in PCB rats was greatly reduced.

Table 1 shows the 17-hr thyroid ^{131}I uptake and serum PBI values in the 3 groups of animals. These values were unchanged in DDT-treated rats. In PCB-treated animals, thyroid ^{131}I uptake was increased (18.7 *vs* 13.5%) and the serum PBI was greatly reduced from a control value of 6.25 to 2.25 μg/100 ml.

Table 2 shows that PCB, administered *in vivo* or added *in vitro* to rat serum, reduced binding of thyroid hormone to serum pro-

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TABLE 1. The effect of polychlorinated biphenyl (PCB) and DDT on serum protein-bound iodine (PBI) and 17-hr thyroid ^{131}I uptake

Treatment	PBI μg/100 ml Mean ± SE	P value	^{131}I uptake % dose Mean ± SE	P value
Control	6.25 ± 0.48	—	13.53 ± 1.43	—
PCB	2.25 ± 0.10	<0.001	19.73 ± 1.78	<0.02
DDT	5.03 ± 0.21	NS	15.18 ± 2.36	NS

Each value is the mean ± SE of 6 determinations.
The statistical significance of the difference between the means was determined by Student's *t* test. NS = not significant.

teins. Control resin uptake of ^{125}I-T_3 was 47% and was increased to over 50% by PCB. PTU treatment, as expected, reduced the resin ^{125}I-T_3 uptake. DDT treatment was without effect.

Discussion

Hepatic conjugation of T_4 to glucuronic acid, catalyzed by UDP-glucuronyltransferase, and excretion in the bile of T_4-glucuronide is a major route of T_4 metabolism (27,28). T_4-glucuronide formation is rate limiting for biliary T_4 excretion (24, 29–31) and the polycyclic hydrocarbon type of hepatic microsomal enzyme inducer (32), such as 3-methylcholanthrene (23) and 3,4-benzpyrene (21) enhance biliary T_4 excretion by increasing hepatic T_4-glucuronide formation.

The effects of PCB resembled those of the polycyclic hydrocarbons (21–23); it increased biliary T_4 excretion 4- to 5-fold, elevated B/P ratios tremendously, enhanced the biliary clearance rate of circulating T_4 and increased the proportion of biliary radioactivity present as T_4-glucuronide, while reducing those present as free T_4 and I^-; PCB probably induced hepatic UDP-glucuronyltransferase. Like phenobarbital (21,32), but unlike the polycyclic hydrocarbons (21–23, 32), it enhanced bile flow.

In PCB-treated rats serum PBI concentrations were less than half of normal. This may be attributed to enhanced biliary excretion of T_4 and also to inhibition of thyroid hormone binding to plasma proteins; resin uptake of serum ^{125}I-T_3 was increased by *in vitro* addition of PCB or by its *in vivo* injection. Inhibition of plasma protein-binding of T_4 may have contributed to the enhancement of biliary T_4 excretion induced by PCB. Salicylate, which also inhibits plasma protein-binding of T_4, accelerated biliary T_4 excretion, but did not alter the proportion of T_4-glucuronide in bile (33). PCB enhanced the disappearance of plasma ^{125}I-T_4 and, secondarily, increased thyroid function, as shown by the elevated thyroid ^{131}I uptake. It seems likely that the goitrogenic effect of PCB in birds (13) was due to enhanced peripheral metabolism of T_4.

DDT belongs to the phenobarbital class of enzyme inducers (32) and the present data in DDT-treated animals resemble those obtained in phenobarbital-treated rats (21), including enhancement of bile flow (21,32). DDT did not significantly increase biliary T_4 excretion and male rats treated with phenobarbital showed a barely significant increase in biliary T_4 excretion (21). Treatment of rats with DDD isomers increased fecal T_4 excretion only after 24 hr had elapsed following ^{131}I-T_4 injection (14). The B/P ratios were consistently higher in DDT-treated rats, reaching significance in the last 2 periods and the biliary clearance rate of plasma T_4 was significantly greater in treated animals. DDT lowered the plasma concentration of ^{125}I-T_4, perhaps by accumulation of T_4 in the liver, such as was reported in chlordane-treated rats (16). Since DDT did not lower the serum PBI, additional T_4 must have been released from

TABLE 2. The effect of PCB and DDT on serum ^{125}I-T_3 uptake by resin

	Treatment	% Resin uptake Mean ± SE	P value
In vivo	Control	47.10 ± 0.57	—
	PCB	53.39 ± 1.16	<0.001
	DDT	47.71 ± 0.71	NS
	PTU	32.22 ± 0.37	<0.001
In vitro	Control	45.58 ± 0.52	—
	PCB	51.51 ± 0.30	<0.001

Each value is the mean ± SE of 6 determinations.
The statistical significance of the difference between the means was determined by Student's *t* test. NS = not significant.

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the thyroid. Hence in treated rats the specific activity of circulating ^{125}I-T_4 may have been lowered and the biliary excretion of ^{127}I-T_4 may have been increased, despite the unchanged excretion of ^{125}I-T_4. DDT increased rat liver bilirubin-glucuronyl-transferase activity (34) and in the present study slightly, but significantly, increased the proportion of T_4-glucuronide in bile. The goitrogenic effect of DDT in birds (11,12) may well have been secondary to enhanced T_4 metabolism.

In man *o, p'*-DDD lowered the serum PBI (35,36) by inhibiting T_4 binding to T_4-binding globulin (36) and exposure to an unnamed organochlorine pesticide reduced serum PBI concentrations (37). However, in rats DDT did not appear to influence plasma protein binding of thyroid hormone; DDT did not affect serum PBI concentrations or the resin uptake of serum ^{125}I-T_3 despite its close structural similarity to PCB (2,5).

Accidental human exposure to large amounts of PCB's causing chloracne and various systemic effects has been reported (5,38). Studies of thyroid function and thyroid hormone degradation in such cases would be of interest.

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