[Chem. Pharm. Bull. 23(4) 831—836 (1975)]

UDC 547.539.2.09 : 577.15.04

Relationship between Chemical Structure and Activity. III.[1] Dose-Response or Time-Course of Induction in Microsomal Enzymes Following Treatment with 1,2,4-Trichlorobenzene[2]

Toshihiko Ariyoshi, Katsumi Ideguchi,[3a] Kazuhide Iwasaki,[3b] and Mitsuo Arakaki[3c]

Faculty of Pharmaceutical Sciences, Nagasaki University[3]

(Received August 5, 1974)

The contents of hepatic constituents and cytochromes as well as the activities of drug-metabolizing enzymes and δ-aminolevulinic acid (δ-ALA) synthetase were determined in rats treated with 1,2,4-trichlorobenzene (1,2,4-TRCB).

1) Liver weight and microsomal protein content were increased in higher doses than 750 mg/kg 24 hr after the administration, but in contrast glycogen content was markedly decreased in higher doses than 500 mg/kg. In a dose of 250 mg/kg and above, the activities of aminopyrine demethylase and aniline hydroxylase, cytochrome P-450 contenn were increased, but on the contrary cytochrome b_5 content showed the tendency to be decreased in high doses. δ-ALA synthetase activity was increased in higher doses than 250 mg/kg, and this increase attained approximately 300% in higher doses than 750 mg/kg.

2) In a single oral dose of 500 mg/kg, glycogen content was decreased markedly from 6 hr after the administration and this decrease was maintained for 48 hr. The increase of microsomal protein content was noted in 3 hr after dosing, but cytochrome P-450 content, activities of aminopyrine demethylase and aniline hydroxylase were increased 24 hr after the administration. These increases were maintained for 5 days, especially in aminopyrine demethylase for 15 days. δ-ALA synthetase activity was decreased markedly in the early stage, that is, 3 or 6 hr after the administration, but increased rapidly after 24 hr.

3) An apparent parallelism between the change in δ-ALA synthetase activity and that in cytochrome P-450 content was not noted.

The wide distributions of chlorinated hydrocarbons throughout foods, water, air and soils, in addition humans, animals and plants have been proved by the results of analysis performed in the world. However, it must be true that these compounds exert various harmful effects on the living animals, although the toxicity caused by accumulation of these organochlorine compounds has not yet been clearly demonstrated. In fact, hexachlorobenzene, one of fungicides, has been shown to be associated with the induction of porphyria in human subjects and experimental animals.[4] Moreover, the followings have been shown: decreased thickness of egg-shells,[5] decreased reproduction in birds,[6] enzyme induction,[7] and so on.

1) Part II: T. Ariyoshi, K.Ideguchi, K.Iwasaki, and M.Arakaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 824 (1975).
2) A part of this work was presented at the 82nd Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, May 1973.
3) Location: *a*) *1-14, Bunkyo-machi, Nagasaki-shi, Nagasaki*; *b*) Present address: *Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 1, Kashima-cho, Higashiyodogawa-ku, Osaka*; *c*) Present address: *Nagasaki Prefectural Womens Junior College, 1007 Narutaki-machi, Nagasaki-shi, Nagasaki.*
4) R.K. Ockner and R. Schmid, *Nature*, **189**, 499 (1961); F. DeMatteis, B.E. Prior, and C. Remington, *Ibid.*, **191**, 363 (1961).
5) D.A. Ratcliffe, *Nature*, **215**, 208 (1967); J.J. Hickey and D.W. Anderson, *Science*, **162**, 271 (1968); J. Bitman, H.C. Cecil, S.J. Harris, and G.F. Fries, *Nature*, **224**, 44 (1969).
6) R.G. Heath, J.W. Spann, and J.F. Kreitzer, *Nature*, **224**, 47 (1969).
7) L.G. Hart, R.W. Schultice, and J.R. Fouts, *Toxicol. Appl. Pharmacol.*, **5**, 371 (1963); F.K. Kinoshita, J.P. Frawley, and K.P. DuBois, *ibid.*, **9**, 505 (1966); J.C. Street and R.W. Chadwick, *ibid.*, **11**, 68 (1967); M. Ikeda, A.H. Conney, and J.J. Burns, *J. Pharmacol. Exptl. Therap.*, **162**, 338 (1968); C.L. Litterst, T.M. Farber, A.M. Baker, and E.J. Van Loon, *Toxicol. Appl. Pharmacol.*, **23**, 112 (1972).

The authors reported in the preceding paper[1] that treatment of rats with 1,2,4-trichlorobenzene (1,2,4-TRCB) in an oral dose of 250 mg/kg once daily for 3 days, caused marked increases of microsomal phospholipid and protein content and an enhancement of drug-metabolizing enzyme activities in addition to a marked increase of cytochrome P-450 content which was partly related to an induction of δ-aminolevulinic acid (δ-ALA) synthetase.

In the present paper, we have further investigated to establish a minimum dose for induction, the rate of enzyme induction by 1,2,4-TRCB and the stability of induced enzymes after a single treatment with 1,2,4-TRCB.

Materials and Methods

1,2,4-TRCB was purchased from Nakarai Chemicals Co. Ltd., Kyoto, Japan. δ-ALA was purchased from Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan. Nicotineamide adenine dimucleotide phosphate (NADP), glucose 6-phosphate disodium salt, glucose 6-phosphate dehydrogenase were purchased from Boehringer Mannheim GmbH, Mannheim, Germany. Aniline and aminopyrine were obtained from commercial sources and used following purification by redistillation and recrystallization, respectively. The other reagents were obtained from commercial sources and used without further purification.

Female Wistar rats weighing 110—140 g were used in all experiments and they were fed commercial rat chow, F-II, Funahashi Nojyo Co. Ltd., Chiba, Japan, for one week prior to the experiments.

1,2,4-TRCB was suspended in 2% tragacanth gum solution and was given orally in a single dose of 500 mg/5 ml/kg. For experiment of dose-response, 1,2,4-TRCB was administered orally in single doses of 125, 250, 500, 750, 1000 and 1500 mg/5 ml/kg, respectively. The control animals received an equal volume of the vehicle orally.

Rats were sacrificed by decapitation 24 hr after the administration except the time-course experiment, and the livers were perfused with ice-cold 0.9% NaCl solution *in situ* to remove blood. The livers were removed and immediately the portions of liver were weighed and used for the direct determination of glycogen[8] and triglyceride.[9] The rest of liver was immediately placed in ice-cold 0.9% NaCl solution. All subsequent procedures were performed below 4°. The livers were homogenized in 3 volumes of 0.25 M sucrose solution containing 1 mM ethylenediaminetetraacetic acid (EDTA) in a motor-driven Potter homogenizer with a Teflon pestle. Preparation of microsomes was carried out by the procedures described previously[10] except microsomal pellets were suspended in the 1.15% KCl-25 mM Tris-HCl, pH 7.4, to give a concentration of 4.50 mg of microsomal protein per ml. Microsomal protein was estimated by the method of Lowry, *et al.*,[11] using bovine serum albumin as a standard. Incubation mixtures consisted of 4.50 mg of microsomal prstein, 5 mM glucose 6-phosphate (G-6-P), 0.5 mM NADP, 1.75 units glucose 6-phosphate dehydrogenase (G-6-PD), 5 mM $MgCl_2$, substrate (1 mM aniline or 2 mM aminopyrine), and 50 mM Tris-buffer, pH 7.4, in a final volume of 3.0 ml. Incubations were carried out at 37° for 10 min aerobically. *p*-Aminophenol formed by aniline hydroxylase was determined by the method of Imai and Sato[12] and formaldehyde formed by aminopyrine demethylase was determined according to Nash reaction.[13] Assay of cytochromes was estimated by the method of Omura and Sato.[14] Aniline-induced difference spectra was obtained by the method of Schenkman, *et al.*[15] The activity of δ-ALA synthetase was incubated as described by Marver, *et al.*[16] and determined by the method of Urata and Granick.[17]

Results

Dose-response Relationship

Various biological parameters were examined 24 hr after treatment of rats with 1,2,4-TRCB in single doses of 125, 250, 500, 750, 1000 and 1500 mg/kg, and the results obtained were summarized in Table I. The body weight was decreased in the highest dose of 1500

8) S. Seifter, S. Dayton, B. Novic, and E. Muntwyler, *Arch. Biochem. Biophys.*, **25**, 191 (1950).
9) W.M. Butler, Jr., H.M. Maling, M.G. Horning, and B.B. Brodie, *J. Lipid Res.*, **2**, 95 (1961).
10) T. Ariyoshi, E. Takabatake, and H. Remmer, *Life Sci.*, **9**, Part II, 361 (1970).
11) O.H. Lowry, N.J. Resebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
12) Y. Imai and R. Sato, *Biochem. Biophys. Res. Commun.*, **25**, 80 (1966).
13) T. Nash, *Biochem. J.*, **55**, 416 (1953).
14) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
15) J.B. Schenkman, H. Remmer, and R.W. Estabrook, *Mol. Pharmacol.*, **3**, 113 (1967).
16) H.S. Marver, D.P. Tschudy, M.G. Perlroth, and A. Collins, *J. Biol. Chem.*, **241**, 1803 (1966).
17) G. Urata and S. Granick, *J. Biol. Chem.*, **238**, 811 (1963).

mg/kg, but a ratio of liver weight to 100 g body weight was increased in higher doses than 500 mg/kg. No change in liver triglyceride content was noted, but, in contrast, a decrease of glycogen content was noted in higher doses than 500 mg/kg, especially in the highest dose. Microsomal protein content was significantly increased in higher doses than 750 mg/kg.

TABLE I. Dose-response Relationship for 1,2,4-Trichlorobenzene on Hepatic Constituents, Cytochromes, Drug-metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

	Control (6)	125 mg/kg (3)	250 mg/kg (3)	500 mg/kg (3)	750 mg/kg (3)	1000 mg/kg (3)	1500 mg/kg (3)
Body weight initial (g)	136±3	139±3	133±2	136±3	142±4	138±2	141±2
final (g)	137±2	137±2	134±2	137±2	142±5	136±3	121±1[a]
Liver weight (g/100 g b.w.)	4.89± 0.12	5.19± 0.05	5.21± 0.08	5.68± 0.11[a]	5.40± 0.20	5.51± 0.21	5.55± 0.03[a]
Glycogen (mg/g liver)	62.9± 3.6	55.0± 3.2	62.4± 3.2	48.6± 3.2[a]	44.3± 3.6[a]	45.2± 2.3	23.8± 0.2[b]
Triglyceride (mg/g liver)	4.1± 0.5	4.1± 1.4	3.9± 0.7	3.5± 0.5	5.2± 0.5	4.2± 0.6	16.6± 5.3
Microsomal protein (mg/g liver)	17.3± 0.4	18.0± 0.3	17.6± 0.6	18.5± 0.4	19.4± 0.4[a]	19.2± 0.3[a]	19.9± 0.4[a]
Cytochromes							
P-450 (nmoles/mg protein)	0.73± 0.04	0.84± 0.05	1.28± 0.06[b]	1.27± 0.10[a]	1.00± 0.09[a]	1.04± 0.13	1.07± 0.02[a]
b_5 (nmoles/mg protein)	0.35± 0.02	0.30± 0.01	0.35± 0.01	0.35± 0.01	0.26± 0.02	0.29± 0.02	0.24± 0.02[a]
Aniline hydroxylase							
formed *p*-aminophenol (nmoles/mg protein/min)	0.53± 0.04	0.60± 0.04	0.76± 0.07[a]	0.81± 0.05[a]	0.91± 0.04[b]	0.81± 0.07[a]	1.26± 0.09[b]
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.73± 0.03	0.71± 0.01	0.59± 0.03	0.64± 0.02	0.91± 0.05[a]	0.78± 0.04	1.18± 0.08[b]
Aminopyrine demethylase							
formed formaldehyde (nmoles/mg protein/min)	3.40± 0.15	4.38± 0.50	5.62± 0.32[a]	5.75± 0.41[a]	5.47± 0.20[a]	4.13± 0.37	4.23± 0.03
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	4.66± 0.13	5.21± 0.46	4.39± 0.03	4.54± 0.15	5.47± 0.48	3.97± 0.13	3.96± 0.06
Spectral change							
aniline-cytochrome P-450 ($E_{430-480}$/mg protein) × 10^3	14.6± 1.1	17.1± 1.4	26.2± 0.2[b]	26.3± 2.2[a]	21.3± 0.8[a]	22.2± 3.1[a]	24.7± 1.0[a]
δ-ALA synthetase(nmoles/g liver/hr)	27.2± 0.9	38.7± 1.6[a]	44.1± 3.9[a]	43.2± 4.7[a]	104.2± 9.9[a]	95.4± 2.0[b]	109.4± 2.5[b]

Rats were given orally 1,2,4-trichlorobenzene at a single dose of 125, 250, 500, 750, 1000 or 1500 mg/kg, and were sacrificed 24 hr after the administration. All values are mean±S.E. of 3 to 6 rats whose number was shown in parenthesis.
a) significantly different from control, $p<0.05$
b) significantly different from control, $p<0.01$

Cytochrome P-450 content was significantly increased in higher doses than 250 mg/kg. No difference was noted in cytochrome b_5 content in lower doses than 500 mg/kg, but the decreased tendency was rather shown in higher doses than 750 mg/kg. The maximal enhancement in activity of aminopyrine demethylase was attained in a dose of 500 mg/kg, but the activity was rather decreased with an increase of the doses. On the other hand, activity of aniline hydroxylase was enhanced with an increase of the doses. The magnitude of difference spectra induced by aniline-cytochrome P-450 binding was increased in higher doses than 250 mg/kg. A significant enhancement was noted in δ-ALA synthetase activity throughout the doses used in this paper.

Time-course after Treatment

In a single dose of 500 mg/kg, biological parameters were examined at 3, 6, 12, 24, 48 hrs and 5, 15 days after treatment of rats with 1,2,4-TRCB, and the results were summarized in

Table II. Effects of a Single Oral Dose of 1,2,4-Trichlorobenzene on Hepatic Constituents, Cytochromes, Drug-metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

Time after administration	Control	3 hr	6 hr	12 hr	24 hr	48 hr
Body weight initial (g)	132±2	138±4	136±2	137±2	133±2	139±3
final (g)	133±2	134±4	130±2	132±1	127±1	122±4
Liver weight (g/100 g b.w.)	4.71± 0.10	4.57± 0.17	4.59± 0.11	4.58± 0.19	4.83± 0.17	5.87± 0.11[a]
Glycogen (mg/g liver)	65.6 ± 3.4	53.9 ± 2.6	35.6 ± 2.7[b]	20.7 ± 2.8[b]	28.0 ± 11.2	44.7 ± 3.4[a]
Triglyceride (mg/g liver)	5.4 ± 0.8	5.5 ± 0.9	6.8 ± 2.8	5.8 ± 0.2	7.1 ± 1.1	8.2 ± 1.5
Microsomal protein (mg/g liver)	16.5 ± 0.5	19.3 ± 0.3[a]	20.4 ± 0.9[a]	20.9 ± 0.6[a]	17.6 ± 0.4	20.7 ± 0.8[a]
Cytochromes						
P-450 (nmoles/mg protein)	0.69± 0.03	0.75± 0.03	0.72± 0.06	0.64± 0.07	0.85± 0.10	1.04± 0.06[a]
b_5 (nmoles/mg protein)	0.26± 0.01	0.30± 0.01	0.26± 0.01	0.23± 0.02	0.25± 0.02	0.28± 0.02
Aniline hydroxylase						
formed *p*-aminophenol (nmoles/mg protein/min)	0.42± 0.04	0.55± 0.03	0.46± 0.06	0.50± 0.07	0.76± 0.04[b]	0.64± 0.04[a]
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.61± 0.04	0.73± 0.07	0.64± 0.05	0.78± 0.07	0.90± 0.10[a]	0.62± 0.01
Aminopyrine demethylase						
formed formaldehyde (nmoles/mg protein/min)	4.24± 0.22	3.93± 0.09	3.85± 0.31	4.04± 0.26	5.66± 0.27[a]	6.88± 0.50[a]
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	6.14± 0.26	5.24± 0.37	5.34± 0.15	6.31± 0.25	6.67± 0.59	6.61± 0.69
Spectral change						
aniline-cytochrome P-450 ($E_{430-480}$/mg protein) × 10^3	11.5 ± 0.7	13.3 ± 0.5	12.4 ± 1.2	12.1 ± 1.9	17.9 ± 1.9[a]	21.3 ± 1.7[b]
δ-ALA synthetase (nmoles/g liver/hr)	39.5 ± 2.9	17.1 ± 0.7[b]	19.0 ± 2.0[b]	43.2 ± 7.2	86.5 ± 8.7[b]	78.1 ± 13.4

Rats were given orally 1,2,4-trichlorobenzene at a single dose of 500 mg/kg, and were sacrificed at the indicated time after the administration. All values are mean±S.E. of 4 rats.
a) significantly different from control, $p<0.05$
b) significantly different from control, $p<0.01$

Table II and III. The liver weight was increased 48 hr after the administration, but no significant difference was noted in triglyceride content at any time after the administration. Glycogen content was markedly decreased 6 hr after the administration and reached a minimum after 12 hr, and thereafter was returned gradually although a significant decrease was still observed after 48 hr. Microsomal protein content showed the tendency to be increased at all measuring time.

Cytochrome P-450 content was increased 3 and 6 hr after the administration and restored to the control levels in 12 hr, and again increased at 24 hr, and this increase was still noted on day 5. No significant difference was noted in cytochrome b_5 content at all measuring time, although the content showed the tendency to be slightly increased on day 5 and 15 after the administration.

The increase in activity of aminopyrine demethylase was noted 24 hr after the administration and still noted on day 15. On the other hand, aniline hydroxylase was enhanced temporarily 3 hr after the administration, and subsequently restored to the control values at 6 and 12 hr, and subsequently a marked enhancement of the activity was caused at 24 hr, which persisted for 5 days.

The magnitude of difference spectrum induced by aniline-cytochrome P-450 binding was increased, and paralleled approximately by the increase of cytochrome P-450 content.

TABLE III. Effects of a Single Oral Dose of 1,2,4-Trichlorobenzene on Hepatic Constituents, Cytochromes, Drug-Metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

	Control (5)	5 days (5)	Control (4)	15 days (4)
Body weight initial (g)	122±2	123±1	115±1	113±2
final (g)	145±2	137±3	173±3	168±3
Liver weight (g/100 g b.w.)	5.15±0.13	5.40±0.22	4.89±0.12	4.62±0.10
Glycogen (mg/g liver)	66.0 ±3.4	63.3 ±4.3	—	—
Triglyceride (mg/g liver)	6.4 ±0.8	6.6 ±0.8	—	—
Microsomal protein (mg/g liver)	16.7 ±0.4	18.3 ±0.4	16.8 ±0.2	18.9 ±0.3
Cytochromes				
P-450 (nmoles/mg protein)	0.69±0.03	0.83±0.02[a)]	0.62±0.03	0.60±0.03
b_5 (nmoles/mg protein)	0.30±0.02	0.32±0.01	0.27±0.02	0.29±0.02
Aniline hydroxylase				
formed *p*-aminophenol (nmoles/mg protein/min)	0.47±0.02	0.62±0.03[a)]	0.39±0.05	0.40±0.02
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.68±0.04	0.75±0.03	0.63±0.07	0.67±0.06
Aminopyrine demethylase				
formed formaldehyde (nmoles/mg protein/min)	4.23±0.33	6.11±0.27[a)]	3.64±0.22	5.32±0.28[a)]
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	6.13±0.34	7.36±0.57	5.88±0.66	8.87±0.64
Spectral change				
aniline-cytochrome P-450 ($E_{430-480}$/mg protein) × 10^3	14.3 ±0.8	17.0 ±0.3	15.5 ±0.6	16.4 ±0.8
δ-ALA synthetase (nmoles/g liver/hr)	34.9 ±3.0	33.0 ±2.4	30.4 ±1.0	28.4 ±2.0

Rats were given orally 1,2,4-trichlorobenzene at a single dose of 500 mg/kg, and were sacrificed at the 5 or 15 days after the administration. All values are mean±S.E. of 4 to 5 rats whose number was shown in parenthesis.
a) significantly different from control, $p<0.05$

δ-ALA synthetase activity was decreased approximately 50% 3 and 6 hr after the administration as compared to the control levels, and subsequently restored to the control levels at 12 hr, and thereafter increased approximately 200% at 24 and 48 hr, and again restored to normal on day 5.

Discussion

In the dose-response experiments, induction phenomena of drug-metabolizing enzymes and cytochrome P-450 were caused by treatment with 1,2,4-TRCB in a single dose of 250 mg/kg. By treatment at the highest dose of 1500 mg/kg, each activity of aminopyrine demethylase and aniline hydroxylase when based on mg protein were enhanced. However, the former was decreased and the latter was increased when each activity was calculated in units of cytochrome P-450. In other words, the change in the content of cytochrome P-450 which is considered to be the terminal oxidase in hepatic microsomal mixed-function oxidations was not always paralleled by the change in the enzyme activity. This may in part be associated with interaction between cytochrome P-450 and remained 1,2,4-TRCB (type I substrate[1)]) in liver microsomes. Because Jondorf *et al.* reported[18)] that 1,2,4-TRCB was metabolized to phenols (42%) and mercapturic acids (0.3%), and was appeared in the urine during five days after dosing. However, no significant difference was noted in the magnitude of difference spectrum induced by hexobarbital (type I substrate)-cytochrome P-450 binding when the treatment with 1,2,4-TRCB at the high doses (1000 mg/kg) or 6 hr after dosing (500 mg/kg).[19)]

18) W.R. Jondorf, D.V. Parke, and R.T. Williams, *Biochem. J.*, **61**, 512 (1955).
19) T. Ariyoshi and Y. Ishizuka: unpublished observation.

Rimington and Ziegler[20] reported that the decrease of liver glutathione content, the increaes of lipid content and the severe liver damage were observed in the treatment with 1,2,4-TRCB at the high doses. In the present study, both body weight and liver glycogen content were decreased at the highest dose, and in addition, we have been reported a marked increase of microsomal phospholipid content by treatment with 1,2,4-TRCB.[1] From the above, it was suggested that the activities of membrane-bound enzymes, such as drug-metabolizing enzyme, would be connected with the morphological and chemical alterations of the membrane.

Although the activity of δ-ALA synthetase, the rate-limiting enzyme in porphyrine biosynthetic pathway,[21] was enhanced with the increase of doses, this change was not paralleled by the change in both content of cytochrome P-450 and b_5 which normally comprised most of the microsomal hemeprotein. It has generally been considered that the induction of δ-ALA synthetase precedes the enhancement of cytochrome P-450.[22] In a time-course experiment after the single administration of 500 mg/kg, however, the cytochrome P-450 content was rather increased in earlier stage, that is, 3 and 6 hr after dosing although δ-ALA synthetase activity was decreased markedly. This phenomenon fairly resembled to the results obtained in the treatment with monochlorobenzene (MCB).[23] However, δ-ALA synthetase activity was enhanced markedly 24 hr after the treatment with MCB, although cytochrome P-450 content was decreased with the elapse of time. On the other hand, 24 hr after the treatment with 1,2,4-TRCB, cytochrome P-450 content was increased rapidly with the enhancement of δ-ALA synthetase activity, and this enhancement was still noted on day 5 after the administration, being accompanied by the enhancement of enzyme activities, that is, the continuation in activities of aniline hydroxylase and aminopyrine demethylase up to day 5 and 15, respectively. These results would be suggested the slow decay of the induced enzyme activities by the treatment with 1,2,4-TRCB.

Although δ-ALA synthetase activity was markedly enhanced by each treatment with MCB and 1,2,4-TRCB the conflicting effects on cytochrome P-450 contents were obtained, it would be anticipated that this conflict might be due to the inhibition of heme biosynthesis or the difference in the degradation of heme. These have still been investigated in our laboratories.

The effects of 1,2,4-TRCB on cytochrome b_5 were different from that on cytochrome P-450. This observation may be explained on the basis of the relatively slow turn-over rate, since a slow turn-over rate would result in a delayed appearance of a response to any changes in the rate of hemeprotein-synthesis.

As the high lipid-soluble organochlorine compounds would be induced the unexpected effects on the living animals *in vivo*, further investigations will be needed from various viewpoints.

Acknowledgement The authors are indebted to Miss M. Etou, Mr. T. Iga and Mr. Y. Ishizuka for carrying out a part of this study.

20) C. Rimington and G. Ziegler, *Biochem. Pharmacol.*, **12**, 1387 (1963).
21) S. Granick and G. Urata, *J. Biol. Chem.*, **238**, 821 (1963); S. Granick, *ibid.*, **241**, 1359 (1966).
22) J. Baron and T.R. Tephly, *Arch. Biochem. Biophys.*, **139**, 410 (1970).
23) T. Ariyoshi, K. Ideguchi, Y. Ishizuka, K. Iwasaki, and M. Arakaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 817 (1975).