Vol. 93, No. 1, 1980
March 13, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 250-257

EFFECT OF CHLORINATED NAPHTHALENES AND TERPHENYLS ON THE ACTIVITIES OF DRUG METABOLIZING ENZYMES IN RAT LIVER

Markku Ahotupa and Antero Aitio

Department of Physiology, University of Turku, SF-20520 Turku 52, Finland

Received January 24,1980

SUMMARY

Chlorinated naphthalenes with high chlorine content, Halowax 1014 and Halowax 1051 (containing 62 and 70 wt.-% Cl, respectively), were potent inducers of enzymes catalyzing drug hydroxylation or glucuronidation in rat liver. A single i.p. dose (100 mg/kg), given 7 days before assaying the enzyme activities, enhanced ethoxycoumarin deethylation 10-fold, benzo(a)pyrene hydroxylation 2 - 5 -fold, and glucuronidation of 4-methylumbelliferone and 2-aminophenol 5-fold. The increases in enzyme activities were dose-dependent. The hydroxylation and glucuronidation reactions achieved maximal rates in one week, and they were still above control levels after one month. Chlorinated terphenyls as well as chloronaphthalenes with low chlorine content enhanced only slightly hydroxylation and glucuronidation reactions. The activities of epoxide hydratase and glutathione S-transferase were not altered after treatment of animals either with chloronaphthalenes or with chloroterphenyls.

Because of their structural relationship, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) and terphenyls (PCTs) have similar chemical and physical properties and the same kinds of industrial applications. In 1970's the world production of PCNs was estimated to be at least 10 % of that of PCBs (1). In addition, PCNs are found as impurities in commercial PCB-mixtures (2,3). PCBs are among the most widespread and persistent environmental pollutants today, and with improved analytical methods also PCNs (4) and PCTs (5) are detected in environmental samples. The levels of PCTs in human tissues in Japan are almost equivalent to PCBs (6).

The fact that many chlorinated aromatic compounds, e.g. TCDD (7), PCBs (8) and DDT (9), are strong inducers of drug metabolizing enzymes suggests that PCNs and PCTs, too, might accelerate drug metabolism. The purpose of the present paper is to describe the effects of PCNs and PCTs on enzymes catalyzing hydroxylation of drugs, metabolism of epoxides and conjugation of xenobiotics with glucuronic acid.

0006-291X/80/050250-08$01.00/0

METHODS

Polychlorinated naphthalenes (Halowax 1031, 1000, 1001, 1099, 1013, 1014 and 1051; Koppers Chemical Co.) and terphenyls (Aroclor 5060, 5442 and 5460; Monsanto Chemical Co.) were purchased from RFR Corporation, Hope, R.I., USA. The chemicals, suspended (Halowax 1051) or dissolved (all other chemicals) in corn oil, were administered to adult male Wistar rats (200-270 g) as an intraperitoneal injection. Microsomes were prepared by the calcium aggregation method (10,11). Glutathione S-transferase activity was determined in the post-microsomal supernatant (12) with reduced volume of acetonitrile, to avoid solvent inhibition of glutathione S-transferase (13). All other enzyme activities were measured with microsomal preparations. The digitonin activation of microsomes was carried out as described by Hänninen (14). Arylhydrocarbon hydroxylase (EC 1.14.14.2) was assayed with fluorometric (15,16) and radiometric (17) methods. Ethoxycoumarin deethylation was determined by a modification (18) of the method of Ullrich and Weber (19). Epoxide hydratase (EC 4.2.1.63) activity was measured with ^{3}H-styrene oxide (20), that of UDP-glucuronosyltransferase (EC 2.4.1.17) with 4-methylumbelliferone (21,22) and 2-aminophenol (23) as substrates. The protein content was estimated by the biuret method (24) with bovine serum albumin as the reference protein. The spectral studies were done with Perkin-Elmer model 555 spectrophotometer.

RESULTS

One week after a single i.p. dose (100 mg/kg) of the chemicals the liver weight of rats treated with Halowax 1013 (56 wt.-% Cl), Halowax 1014 (62 wt.-% Cl), and Halowax 1051 (70 wt.-% Cl), were increased 19 %, 21 % and 24 % ($2P > 0.05$), respectively. Two chloronaphthalenes with the highest chlorine content, Halowax 1014 and 1051, enhanced the activity of arylhydrocarbon hydroxylase 2-fold when measured with the fluorimetric method and 5-fold when measured with the radiometric method; the activity of ethoxycoumarin deethylase increased 10-fold. Other chloronaphthalenes and chloroterphenyls caused only minor, if any, changes in hydroxylation reaction rates (Table 1). No changes were detected in the activities of epoxide hydratase and glutathione S-transferase in animals treated with any of the chloronaphthalenes or chloroterphenyls (Table 1). Halowax 1014 and 1051 increased the glucuronidation of 4-methylumbelliferone and 2-aminophenol 5-fold, both in native and in digitonin activated microsomes (Table 2). After administration of Halowax 1014 and 1051 the absorption maximum of the reduced CO-difference spectrum was at 448.5 nm and 447.5 nm, respectively.

Induction of hydroxylation and glucuronidation reactions by Halowax 1014 and 1051 was dose-dependent. A single dose of 10 mg/kg, given 7 days before

TABLE 1. Effect of commercial mixtures of chloronaphthalenes and chloroterphenyls on the activities of mixed function oxidases and epoxide metabolizing enzymes in rat liver[a]

	Chlorine content[b]	Benzo(a)pyrene hydroxylation (fluorometric assay)[c]	Benzo(a)pyrene hydroxylation (radiometric assay)[c]	Ethoxycoumarin deethylation[c]	Styrene oxide hydration	Conjugation of styrene oxide with glutathione[d]
Control		0.206 ± 0.037[e]	5.64 ± 0.88	9.19 ± 1.13	110 ± 18	102 ± 8
Halowax 1031	22	0.210 ± 0.012	8.10 ± 0.83	14.2 ± 2.1[f]	82.6 ± 7.7	105 ± 18
Halowax 1000	26	0.158 ± 0.025	3.07 ± 0.90	9.11 ± 2.36	116 ± 15	93.0 ± 10.6
Halowax 1001	50	0.192 ± 0.013	3.12 ± 0.50	9.26 + 2.04	141 ± 14	95.2 ± 7.1
Halowax 1099	52	0.200 ± 0.048	8.28 ± 1.12	15.5 ± 1.6[g]	79.4 ± 11.3	109 ± 14
Halowax 1013	56	0.207 ± 0.035	10.9 ± 1.7[f]	20.0 ± 2.3[h]	97.6 ± 10.8	94.0 ± 5.7
Halowax 1014	62	0.433 ± 0.071[g]	30.5 ± 4.4[h]	90.3 ± 9.2[h]	120 ± 19	107 ± 15
Halowax 1051	70	0.438 ± 0.050[g]	31.1 ± 2.0[h]	92.8 ± 9.7[h]	129 ± 11	106 ± 10
Aroclor 5060		0.163 ± 0.018	3.71 ± 0.57	8.81 ± 2.95	109 ± 22	90.8 ± 8.3
Aroclor 5442		0.296 ± 0.072	8.76 ± 1.92	34.2 ± 12.0[f]	172 ± 51	97.7 ± 10.8
Aroclor 5460		0.182 ± 0.023	3.31 ± 0.75	9.62 ± 2.41	130 ± 15	98.7 ± 10.0

[a] Chloronaphthalenes and chloroterphenyls were given as a single i.p. dose (100 mg/kg body weight) 7 days before assaying the enzyme activities.

[b] wt.-% Cl as given by Brinkman and Reymer (26).

[c] nmol/min x g wet weight.

[d] nmol/min x mg prot.

[e] Mean ± SEM from 4 animals.

[f] $2P < 0.05$ by Student's t-test.

[g] $2P < 0.01$ by Student's t-test.

[h] $2P < 0.001$ by Student's t-test.

TABLE 2. Effect of mixtures of chloronaphthalenes and chloroterphenyls on UDPglucuronosyltransferase activity in rat liver[a]

	Chlorine content	4-methylumbelliferone glucuronidation[a]		o-aminophenol glucuronidation[c]	
		native microsomes	digitonin-activated microsomes	native microsomes	digitonin activated microsomes
Control		24.4 ± 2.1[d]	335 ± 26	0.818 ± 0.098	3.05 ± 0.30
Halowax 1031	22	33.1 ± 2.0[e]	529 ± 44[f]	1.10 ± 0.08	3.84 ± 0.38
Halowax 1000	26	33.4 ± 1.5[e]	346 ± 23	0.888 ± 0.163	3.18 ± 0.34
Halowax 1001	50	35.4 ± 3.3[e]	352 ± 23	1.08 ± 0.08	3.72 ± 0.13
Halowax 1099	52	37.0 ± 3.2[f]	629 ± 41[g]	1.33 ± 0.13[e]	6.78 ± 0.54[g]
Halowax 1013	56	34.0 ± 2.7[e]	484 ± 57[e]	1.13 ± 0.13	5.05 ± 0.45[f]
Halowax 1014	62	113 ± 6[g]	1430 ± 100[g]	3.20 ± 0.46[g]	17.4 ± 2.1[g]
Halowax 1051	70	117 ± 9[g]	1650 ± 180[g]	3.78 ± 0.08[g]	22.0 ± 3.0[g]
Aroclor 5060		26.0 ± 2.0	288 ± 6	0.710 ± 0.073	2.60 ± 0.19
Aroclor 5442		40.2 ± 4.4[f]	549 ± 141	1.24 ± 0.21	5.52 ± 1.23[e]
Aroclor 5460		34.8 ± 1.4[f]	408 ± 31	0.843 ± 0.064	3.27 ± 0.25

[a] Chloronaphthalenes and chloroterphenyls were given as a single i.p. dose (100 mg/kg body weight) 7 days before assaying the enzyme activities.

[b] Wt.-% Cl as given by Brinkman and Reymer (26).

[c] nmol/min x g wet weight.

[d] Mean ± SEM from 4 animals.

[e] $2P < 0.05$ by Student's t-test.

[f] $2P < 0.01$ by Student's t-test.

[g] $2P < 0.001$ by Student's t-test.

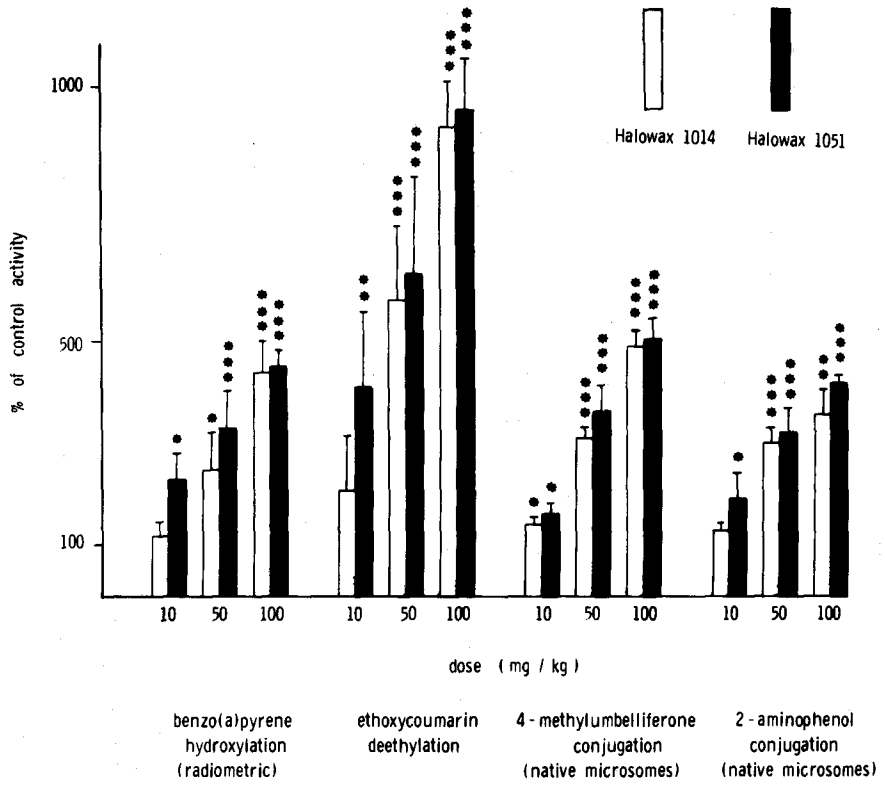

Figure 1. Effect of various doses of highly chlorinated naphthalenes on the activities of enzymes catalyzing drug hydroxylation or glucuronidation in rat liver. Enzyme activities were measured 7 days after a single ip dose. The number of animals was 4. The significance is expressed as follows: * = $2P < 0.05$, ** = $2P < 0.01$, and *** = $2P < 0.001$ by Student's t-test.

assaying the enzyme activities, was sufficient to enhance hydroxylation and glucuronidation reactions. At low doses (10 mg/kg) Halowax 1051, the chloronaphthalene with the highest chlorine content, enhanced hydroxylation reactions more than did Halowax 1014 (Figure 1).

After a single dose (50 mg/kg) of Halowax 1051 the hydroxylation and glucuronidation reactions achieved maximal rates in 1 week, whereafter the activities began to decline. However, after one month the activities were still above control levels (Figures 2 and 3).

DISCUSSION

In the present study it was found that highly chlorinated naphthalenes of the Halowax series are potent inducers of enzymes catalyzing drug hydroxyl-

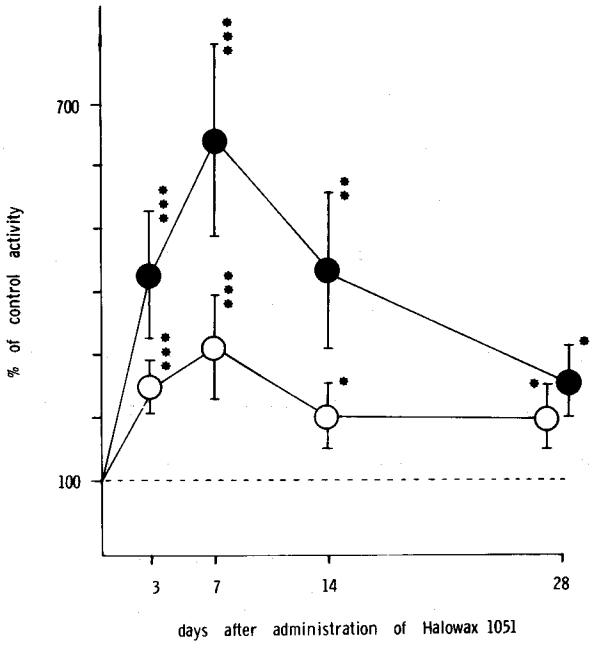

Figure 2. Time profiles of arylhydrocarbon hydroxylase (open circles) and ethoxycoumarin deethylase (closed circles) activities in rat liver after a single ip dose (50 mg/kg) of Halowax 1051. The number of animals was 4 - 6. The significance is expressed as follows:
* = 2P < 0.05, ** = 2P < 0.01, and *** = 2P < 0.001.

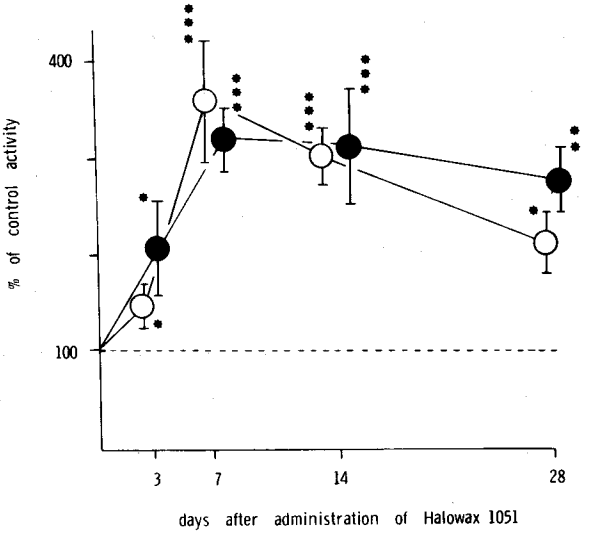

Figure 3. Time profile of UDPglucuronosyltransferase activity after a single ip dose (50 mg/kg) of Halowax 1051. The activity was measured with 4-methylumbelliferone (open circles) and 2-aminophenol (closed circles) as the aglycone. The number of animals was 4 - 6. The significance is expressed as follows:
* = 2P < 0.05, ** = 2P < 0.01, and *** = 2P < 0.001.

ation or glucuronidation. The chlorine content of Halowaxes apparently determined their inducing capacity: the strongest inductions were seen in animals treated with the most chlorine containing Halowax 1051. Similarly, the toxicity of PCNs increase with increasing chlorine content (25). The same kind of relationship between the chlorine content and inducing capacity is described also for the PCBs (8). The chlorine content of Halowax 1014 was only 6 % higher than that of Halowax 1013, yet there was a striking difference in the inducing capacity of these chloronaphthalene-mixtures. This could be explained by the different composition of these mixtures: Halowax 1014 consists principally of hexa- and pentachloronaphthalenes, Halowax 1013 of penta- and tetrachloronapthalenes (26).

Comparison of the results of fluorimetric and radiometric arylhydrocarbon hydroxylase assays (27) reveals that the total metabolism of benzo(a)pyrene increased twice as much as the 3- and 9-hydroxylation in hepatic microsomes of rats treated with highly chlorinated naphthalenes. This indicates that PCN-treatment brings about a qualitative change in the metabolism of benzo(a)-pyrene.

The activities of epoxide hydratase and glutathione S-transferase were not induced by chloronaphthalenes, neither are they induced by TCDD (28). The lack of induction is particularly noteworthy because the enzymes are able to detoxify active intermediates produced by mixed function oxidases. In this respect PCNs and TCDD differ from PCBs which, in addition to drug hydroxylation and glucuronidation, also enhance the metabolism of epoxides (29).

Cornish and Block (30) have shown that PCNs with high chlorine content (penta- to octachloronaphthalenes), in contrast to choloronaphthalenes with low chlorine content, do not yield urinary metabolites suggesting that they are accumulated in the body. This could be the reason for the elevated enzyme activities still 4 weeks after a single dose of Halowax 1051 seen in the present study.

The exact composition of Halowaxes, in regard to components other than chloronaphthalenes, is not known. Therefore the possibility cannot be excluded

that the enhanced enzyme activities were due to contaminating impurities.

The molecular structure of polychlorinated terphenyls, with three subsequent benzene rings, differs essentially from that of the well known chloroaromatics with strong inducing capacity. The present study demonstrated that PCTs affect the activities of drug metabolizing enzymes only slightly.

ACKNOWLEDGEMENTS: This research was financially supported by the Finnish Academy of Sciences, and by NIH Grant AM06018. AA. was recipient of Senior Researchers Grant of the Academy of Finland.

REFERENCES

1. Beland, F.A., and Geer, R.D. (1973) J.Chromatogr., 84, 59-65
2. Vos, J.G., Koeman, J.H., Van der Maas, H.L., Ten Noever de Brauw, M.C., and de Vos, R.H. (1970) Fd Cosmet.Toxicol., 8, 625-633
3. Roach, J.A.G., And Pomerantz, I.H. (1974) Bull.Environ.Contam.Toxicol., 12, 338-342
4. Minagawa, K., Takizawa, Y., Kitajima, T., and Toda, Y. (1976) Jap.J.Ind. Health, 18, 416-417
5. Zitko, V., Hutzinger, O., Jamieson, W.D., and Choi, P.M.K. (1972) Bull. Environ.Contam.Toxicol., 7, 200-201
6. Doguchi, M. (1977) Ecotoxicology and environmental safety, 1, 239-248
7. Lucier, G.W., McDaniel, O.S., Hook, G.E.R., Fowler, B.A., Sonawane, B.R., and Faeder, E. (1973) Environ.Health Perspect., 5, 199-209
8. Ecobichon, D.J., and Comeau, A.M. (1974) Chem.-Biol.Interactions, 9, 341-350
9. Sell, J.L., and Davison, K.L. (1973) Federations Proceedings, 32, 2003-2009
10. Kamath, S.A., and Narayan, K.A. (1972) Anal.Biochem., 48, 59-61
11. Aitio, A., and Vainio, H. (1976) Acta Pharmacol. et toxicol., 39, 555-561
12. James, M.O., Fouts, J.R., and Bend, J.R. (1976) Biochem.Pharmacol., 25, 187-195
13. Aitio, A., and Bend, J.R. (1979) FEBS Letters, 101, 187-190
14. Hänninen, O. (1968) Ann.Acad.Sci.Fenn.Ser. A 11, 142, 1-96
15. Nebert, D.W., and Gelboin, H.V. (1968) J.Biol.Chem., 243, 6250-6261
16. Vainio, H. (1973) Xenobiotica, 3, 715-725
17. DePierre, J.W., Moron, M.S., Johannesen, K.A.M., and Ernster, L. (1975) Anal.Biochem., 63, 470-484
18. Aitio, A. (1978) Anal.Biochem., 85, 488-491
19. Ullrich, V., and Weber, P. (1972) Hoppe-Seyler's Z.Physiol.Chem., 353, 1171-1177
20. Oesch, F., Jerina, D.M., and Daly, J. (1971) Biochim.Biophys.Acta, 227, 685-691
21. Arias, I.M. (1962), J.Clin.Invest., 41, 2233-2245
22. Aitio, A. (1974) Int.J.Biochem., 5, 617-621
23. Dutton, G.J., and Storey, I.D.E. (1962) Methods in Enzymology, vol. 5, pp. 159-164, Acadmic Press, New York
24. Layne, E.K. (1957) Methods in Enzymology, vol. 3, pp. 447-454, Academic Press, New York
25. Bell, W.B. (1953) Vet.Med., 48, 135-140
26. Brinkman, U.A.,Th., and Reymer, H.G.M. (1976) J.Chromatogr., 127, 203-243
27. Holder, G., Yagi, H., Levin, W., Lu, A.Y.H., and Jerina, D.M. (1975) Biochem.Biophys.Res.Commun., 65, 1363-1370
28. Aitio, A., and Parkki, M.G. (1978) Toxicol.Appl.Pharmacol., 44, 107-114
29. Parkki, M.G., Marniemi, J., and Vainio, H. (1977) J.Toxicol.Environ. Health, 3, 903-911
30. Cornish, H.H., and Block, W.D. (1958) J.Biol.Chem., 231, 583-588