was tested in 6 animals. The carotid bifurcation concerned had been previously isolated and was perfused with arterial blood (45 ml/min) by means of a perfusion pump interposed in the common carotid artery. During airway occlusion for a 90-sec period, the perfusion pressure increased from 35±5 mm mercury to 42±5 mm mercury (mean±SE) significantly ($p < 0.01$). Figure 2 shows a typical example obtained in a single experiment. After a latency of about 15 or 20 sec, the pressure rise started and continued throughout the occlusion period.

In some animals, the neurogram of peripheral CSN was recorded under identical conditions. In these cases, the carotid bifurcation was perfused with a larger volume (90 ml/min) in order to make the intravasal pressure exceed the threshold as well as to keep the pressure consistent. Before and after airway occlusion, the afferent discharges of the CSN occurred spontaneously but less frequently (figure 3). In contrast, a few seconds after airway occlusion had started, bursts of mass discharges appeared with a tendency to become larger over the entire occlusion period. These bursts coincided with the periods of efferent activity in the neurogram of both the phrenic nerve and the CN. This fact can be seen by comparing figure 3 with figure 1, especially by comparison of the time related arrangement of these bursts with the simultaneous change in blood pressure and heart rate.

Consequently, breathing seems to be capable of influencing the baroreceptor function by

a) modulating the phasic quantity of ESA that is led to the carotid bifurcation and may make the nervous receptor elements subthreshold for each of these small time periods, and

b) modulating the tonic quantity of ESA that alters the vessel wall stiffness.

1 Present address: Institute of Physiology, Friedrich Schiller University, DDR-69 Jena, German Democratic Republic.
2 W.R. Loewenstein, in: Handbook of Sensory Physiology, vol. 1. Springer-Verlag, Berlin 1971.
3 W.T. Catton, Physiol. Rev. *50*, 297 (1970).
4 R.J. Bagshaw and L.H. Peterson, Am. J. Physiol. *222*, 1462 (1972).
5 K. Koizumi and A. Sato, Am. J. Physiol. *216*, 321 (1969).
6 S.R. Sampson and E. Mills, Am. J. Physiol. *218*, 1650 (1970).
7 A.S. Paintal, Pharmac. Rev. *16*, 341 (1964).
8 F. Palme, Z. exp. Med. 415 (1944).
9 D.J. Reis and K. Fuxe, Am. J. Physiol. *215*, 1054 (1968).
10 P.M. Gootman and M.I. Cohen, in: Central-Rhythmic and Regulation. Hippocrates-Verlag, Stuttgart 1974.

Brominated benzene induction of hepatic porphyria[1]

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Summary. Unlike the highly porphyrinogenic fungicide hexachlorobenzene, hexabromobenzene was a poor inducer of porphyria. Similarly, 1,2-dibromobenzene and 1,2,4-tribromobenzene, while causing small increases in hepatic porphyrins, did not increase ALA synthetase or the urinary excretion of porphobilinogen (PBG), aminolevulinic acid (ALA) or porphyrins.

The porphyrinogenic effects of the fungicide hexachlorobenzene have been observed in a number of laboratory species[2-5] and in humans[6,7]. The less chlorinated benzenes cause much less porphyria[8,9]. Since brominated compounds are often times more active than the chlorinated analogs, it was important to investigate the effects of the fire retardant hexabromobenzene (HBB) on porphyrin synthesis. Mendoza et al.[10] found that in the male rat feeding of HBB at levels up to 160 ppm for 12 weeks did not result in porphyria although there was a non-significant tendency toward increased liver porphyrins. It was also of interest to examine the effects of 2 less halogenated benzenes, 1,4-dibromobenzene and 1,2,4-tribromobenzene. In the induction of xenobiotic metabolism these 2 compounds were more potent than the chlorinated analogs[11]. Dibromobenzene has been identified in drinking water in the United States[12].

Materials and methods. Groups of 5 female (known to be more susceptible than males to the porphyrinogenic effects of hexachlorobenzene[2,13]) Sprague-Dawley derived rats (Laboratory Supply, Indianapolis, IN) were administered 50, 100 or 200 mg/kg of 1,4-dibromobenzene, 1,2,4-tribromobenzene or hexabromobenzene in corn oil daily p.o. for 30, 60, 90 or 120 days. Controls received corn oil (1% v/w). Immediately after the last dose, the rats were placed in metabolism cages for the collection of 24 h urine samples. The animals were sacrificed, livers removed and portions prepared as 33⅓% homogenates in 0.9% NaCl containing 0.5 mM EDTA and 10 mM tris buffer pH 7.2. Samples were precipitated with 0.3 M trichloroacetic acid and centrifuged. Urine samples or liver supernatants were passed through 'piggy-back' ion exchange columns[14] and separations carried out[15] by washing with water and eluting the anionic column once with 2 ml of 1.0 N acetic acid and 2.0 ml of 0.2 N acetic acid for porphobilinogen (PBG) measurement[16]. The column was then washed with 8 ml of 1.5 N HCl and porphyrins measured fluorometrically (excitation at 400 nm and emission at 600 nm) with coproporphyrin as the standard. The cationic column was eluted with 7 ml of 1 M sodium acetate and delta-aminolevulinic acid (ALA) determined[14]. For the livers, only porphyrin content was measured. Portions of the liver homogenate were also used to measure ALA synthetase activity using the procedure of Marver et al.[17] as modified by Goldstein et al.[18] Comparisons among the dose levels were made using Duncan's new multiple range test[19].

Results and discussion. Despite the ability of hexachlorobenzene to increase the production and excretion of porphyrins many-fold[3-5,9], hexabromobenzene had very little effect (table 1). Even at a dose of 200 mg/kg daily for 120 days there was only a 42% increase in porphyrin content of the liver and no increased excretion in the urine. No increases were observed in ALA synthetase or the excretion of ALA or PBG in the urine.

1,4-Dibromobenzene also was not very porphyrinogenic (table 2). The highest increase in liver porphyrin content was approximately 2-fold. In this regard it was slightly more potent than the chlorine derivative[9]. If one considers the enlargement of the liver, the increase is amplified. There was no increase in the urinary excretion of porphyrins, PBG or ALA or in the hepatic synthesis of ALA.

The administration of 1,2,4-tribromobenzene caused a sig-

Table 1. Effect of hexabromobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (μg/24 h)
	30 days of administration		
0	7.56 ± 0.42^{a}	305 ± 13^{a}	1.95 ± 0.07^{a}
50	8.23 ± 0.14^{a}	364 ± 12^{b}	1.35 ± 0.08^{b}
100	8.16 ± 0.19^{a}	382 ± 25^{b}	1.99 ± 0.28^{a}
200	7.36 ± 0.24^{a}	$348 \pm 11^{a,b}$	1.50 ± 0.13^{b}
	60 days of administration		
0	6.98 ± 0.18^{a}	344 ± 36^{a}	1.10 ± 0.18^{a}
50	7.28 ± 0.31^{a}	662 ± 28^{b}	1.08 ± 0.11^{a}
100	7.38 ± 0.43^{a}	535 ± 38^{b}	0.87 ± 0.07^{a}
200	7.36 ± 0.19^{a}	624 ± 78^{b}	0.92 ± 0.14^{a}
	90 days of administration		
0	$7.43 \pm 0.33^{a,b}$	574 ± 20^{a}	1.97 ± 0.43^{a}
50	6.85 ± 0.20^{a}	815 ± 108^{b}	2.00 ± 0.23^{a}
100	7.80 ± 0.23^{b}	801 ± 61^{b}	1.78 ± 0.22^{a}
200	$7.32 \pm 0.20^{a,b}$	798 ± 81^{b}	2.81 ± 0.40^{a}
	120 days of administration		
0	7.95 ± 0.38^{a}	497 ± 26^{a}	2.08 ± 0.30^{a}
50	$7.21 \pm 0.25^{a,b}$	552 ± 36^{a}	2.01 ± 0.35^{a}
100	$8.00 \pm 0.34^{a,b}$	694 ± 51^{b}	1.76 ± 0.27^{a}
200	8.78 ± 0.52^{b}	705 ± 47^{b}	2.19 ± 0.48^{a}

[a] and [b] Values with same superscript are not significantly different ($p > 0.05$).

Table 2. Effect of 1,4-dibromobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (μg/24 h)
	30 days of administration		
0	7.72 ± 0.54^{a}	437 ± 46^{a}	1.48 ± 0.11^{a}
50	7.30 ± 0.28^{a}	506 ± 46^{a}	1.32 ± 0.17^{a}
100	$8.36 \pm 0.20^{a,b}$	531 ± 54^{a}	1.81 ± 0.04^{a}
200	9.34 ± 0.31^{b}	574 ± 59^{a}	1.75 ± 0.39^{a}
	60 days of administration		
0	7.37 ± 0.33^{a}	416 ± 46^{a}	1.89 ± 0.31^{a}
50	$8.11 \pm 0.14^{a,b}$	467 ± 33^{a}	$1.36 \pm 0.20^{a,b}$
100	$8.97 \pm 0.15^{b,c}$	482 ± 55^{a}	1.12 ± 0.08^{b}
200	9.30 ± 0.49^{c}	534 ± 37^{a}	$1.60 \pm 0.25^{a,b}$
	90 days of administration		
0	6.58 ± 0.38^{a}	295 ± 12^{a}	1.33 ± 0.17^{a}
50	8.02 ± 0.36^{b}	405 ± 9^{b}	1.13 ± 0.18^{a}
100	9.81 ± 0.22^{c}	445 ± 20^{b}	1.65 ± 0.48^{a}
200	10.03 ± 0.38^{c}	603 ± 32^{c}	1.40 ± 0.14^{a}
	120 days of administration		
0	7.98 ± 0.38^{a}	301 ± 24^{a}	2.08 ± 0.30^{a}
50	$8.97 \pm 0.45^{a,b}$	417 ± 19^{b}	2.01 ± 0.35^{a}
100	$10.29 \pm 0.27^{b,c}$	436 ± 10^{b}	1.76 ± 0.27^{a}
200	11.32 ± 0.67^{c}	518 ± 57^{b}	2.19 ± 0.48^{a}

[a-c] Values with same superscript are not significantly different ($p > 0.05$).

nificant increase in liver weight even at the lowest level of 50 mg/kg in 30 days (table 3). However, it was not until 90 days that there was an increase in porphyrin content in the liver. The increases were dose dependent but were small and very similar to those which were observed with 1,2,4-trichlorobenzene[9]. As with the other compounds in this study, increased production of porphyrins was not accompanied by an increase in excretion, and there were no increases in hepatic ALA synthetase activity or the urinary excretion of PBG or ALA. The animals treated with 200 mg/kg of 1,2,4-tribromobenzene for 120 days appeared to be slightly smaller than the controls, but the difference was not significant ($p > 0.05$). They were discolored and the ears were especially brown and ragged in appearance.

Table 3. Effect of 1,2,4-tribromobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (μg/24 h)
	30 days of administration		
0	6.46 ± 0.34^{a}	526 ± 49^{a}	2.19 ± 0.28^{a}
50	8.94 ± 0.56^{b}	602 ± 90^{a}	2.29 ± 0.69^{a}
100	8.94 ± 0.56^{b}	620 ± 66^{a}	2.40 ± 0.13^{a}
200	9.52 ± 0.30^{b}	471 ± 22^{a}	2.53 ± 0.27^{a}
	60 days of administration		
0	7.30 ± 0.21^{a}	156 ± 64^{a}	1.92 ± 0.58^{a}
50	9.16 ± 0.42^{b}	251 ± 75^{a}	1.61 ± 0.21^{a}
100	9.96 ± 0.37^{b}	285 ± 78^{a}	1.77 ± 0.48^{a}
200	9.72 ± 0.50^{b}	285 ± 54^{a}	2.42 ± 0.54^{a}
	90 days of administration		
0	6.44 ± 0.26^{a}	468 ± 12^{a}	$1.41 \pm 0.23^{a,b}$
50	8.97 ± 0.43^{b}	629 ± 20^{b}	1.17 ± 0.18^{a}
100	10.07 ± 0.18^{b}	700 ± 17^{c}	2.43 ± 0.59^{b}
200	12.20 ± 0.88^{c}	711 ± 26^{c}	$1.88 \pm 0.35^{a,b}$
	120 days of administration		
0	7.27 ± 0.38^{a}	411 ± 13^{a}	1.75 ± 0.39^{a}
50	11.11 ± 0.80^{b}	560 ± 33^{b}	1.81 ± 0.42^{a}
100	12.71 ± 0.84^{b}	$646 \pm 28^{b,c}$	2.27 ± 0.56^{a}
200	15.18 ± 0.23^{c}	682 ± 45^{c}	2.13 ± 0.40^{a}

[a-c] Values with same superscript are not significantly different ($p > 0.05$).

Thus, although the animals did not appear healthy, porphyria did not appear to be much of a problem.

The results indicated that hexabromobenzene does not share the extreme porphyrinogenic properties of its chlorinated analog. The less brominated compounds are similar to the chlorinated analogs in being very weak in their ability to induce porphyria. Thus the data would indicate that, unlike hexachlorobenzene, porphyria would not be a significant hazard associated with the intake of these compounds.

1 Supported by grant R805070 from the US Environmental Protection Agency.

2 C. San Martin de Viale, A.A. Viale, S. Nacht and M. Grinstein, Clinica chim. Acta *28*, 13 (1970).
3 J.J.F. Taljaard, B.C. Shanley, W.M. Deppe and S.M. Joubert, Br. J. Haemat. *23*, 587 (1972).
4 C. Rajamanickam, J. Amrutavalli, M.R.S. Rao and C. Padmanaban, Biochem. J. *129*, 381 (1972).
5 M.D. Stonard, Br. J. Haemat. *27*, 617 (1974).
6 C. Cam and G. Nigogosyan, J. Am. med. Ass. *183*, 88 (1963).
7 R.K. Ockner and R. Schmid, Nature *189*, 499 (1961).
8 C. Rimington and G. Ziegler, Biochem. Pharmac. *12*, 1387 (1963).
9 G.P. Carlson, Experientia *33*, 1627 (1977).
10 C.E. Mendoza, H.M. Vijay, J.B. Shields and G.W. Laver, Toxic. appl. Pharmac. *41*, 127 (1977).
11 G.P. Carlson, Ann. N.Y. Acad. Sci. *298*, 159 (1977).
12 R.G. Tardiff Compilation of organic compounds identified in drinking waters in the United States as of April 1, 1976. Health Effects Research Laboratory, US Environmental Protection Agency, Cincinnati, Ohio.
13 D.L. Grant, F. Iverson, G.V. Hatina and D.C. Villeneuve, Envir. Physiol. Biochem. *4*, 159 (1974).
14 V.M. Doss and A. Schmidt, Z. klin. Chem. Biochem. *9*, 99 (1971).
15 W.N. Piper, L.W. Condie and T.R. Tephly, Archs Biochem. Biophys. *159*, 671 (1973).
16 D. Mauzerall and S. Granick, J. biol. Chem. *219*, 435 (1956).
17 H.S. Marver, D.P. Tschudy, M.G. Perlroth, A. Collins and G. Hunter, Jr, J. biol. Chem. *241*, 2803 (1966).
18 J.A. Goldstein, P. Hickman and D.L. Jue, Toxic. appl. Pharmac. *27*, 437 (1974).
19 R.G.D. Steel and J.H. Torrie, in: Principles and Procedures of Statistics, p. 107, McGraw-Hill, New York 1960.