Darbishire, F. V. & Thorpe, J. F. (1905). *J. chem. Soc.* **87**, 1717.
Dische, R. & Rittenberg, D. (1954). *J. biol. Chem.* **211**, 199.
Elliott, T. H., Parke, D. V. & Williams, R. T. (1959). *Biochem. J.* **72**, 193.
Emmrich, R., Neumann, P. & Emmrich-Glaser, I. (1941). *Hoppe-Seyl. Z.* **267**, 228.
Feigl, F. (1956). *Spot Tests in Organic Analysis*, 5th ed. London: Elsevier Publishing Co. Ltd.
Fellows, J. K., Luduena, F. P. & Hanzlik, P. J. (1947). *J. Pharmacol.* **89**, 210.
Gessner, P. K. (1958). Ph.D. Thesis: University of London.
Gessner, P. K. & Williams, R. T. (1959). *Pracov. Lék.* **11**, 164.
Gibson, D. M., Titchener, E. B. & Wakel, S. J. (1958). *Biochim. biophys. Acta*, **30**, 376.
Green, D. E., Dewan, J. G. & Leloir, L. F. (1937). *Biochem. J.* **31**, 934.
Hobbs, D. C. & Koeppe, R. E. (1958). *J. biol. Chem.* **230**, 655.
Kamil, I. A., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 235.
Lee, J. S. & Lifson, N. (1951). *J. biol. Chem.* **193**, 253.
Ludwig, B. J. & Piech, E. C. (1951). *J. Amer. chem. Soc.* **73**, 5779.
Matignon, C., Moureu, H. & Dodé, M. (1935). *Bull. Soc. chim. Fr.* [5], **2**, 1181.
Mead, J. A. R., Smith, J. N. & Williams, R. T. (1958). *Biochem. J.* **68**, 61.
Miura, S. (1911). *Biochem. Z.* **36**, 25.
Neubauer, O. (1901). *Arch. exp. Path. Pharmak.* **46**, 133.
Neuberg, C. & Gottschalk, A. (1925). *Biochem. Z.* **162**, 484.
Rothstein, M. & Miller, L. L. (1952). *J. biol. Chem.* **199**, 199.
Rudney, H. (1950). *Arch. Biochem.* **29**, 231.
Smyth, H. F., jun., Seaton, J. & Fischer, L. (1941). *J. industr. Hyg.* **23**, 259.
Thierfelder, H. & Mering, J. von (1885). *Hoppe-Seyl. Z.* **9**, 511.
Weitzel, G. (1947*a*). *Hoppe-Seyl. Z.* **282**, 174.
Weitzel, G. (1947*b*). *Hoppe-Seyl. Z.* **282**, 185.
Wessely, R. (1901). *Mh. Chem.* **22**, 66.
Westerfeld, W. W. & Berg, R. L. (1943). *J. biol. Chem.* **148**, 523.
Wojcik, B. & Adkins, H. (1933). *J. Amer. chem. Soc.* **55**, 4943.

Studies in Detoxication

81. THE METABOLISM OF HALOGENOBENZENES: (*a*) PENTA- AND HEXA-CHLOROBENZENES. (*b*) FURTHER OBSERVATIONS ON 1:3:5-TRICHLOROBENZENE

BY D. V. PARKE AND R. T. WILLIAMS
Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(*Received* 11 *June* 1959)

The metabolic fate of mono-, di-, tri- and tetra-chlorobenzene has already been reported (Smith, Spencer & Williams, 1950; Azouz, Parke & Williams, 1955; Parke & Williams, 1955; Jondorf, Parke & Williams, 1955, 1958). These studies suggested that the more chlorine the halogenated benzene contained, the less readily was it metabolized. To complete the series of chlorinated benzenes, an investigation of the fate of penta- and hexa-chlorobenzene in rabbits has been carried out. Pentachlorobenzene is only slightly altered *in vivo*, and hexachlorobenzene seems to be metabolically inert. Studies of these highly chlorinated benzenes have been carried out in connexion with work in this Laboratory on the metabolism of the highly chlorinated insecticides, dieldrin and aldrin, which also appear to be metabolically inert. Pentachlorobenzene does not have any important practical application, but hexachlorobenzene has limited use as a fungicidal agent for seeds.

Jondorf *et al.* (1955) were unable to account for the major portion of the dose of 1:3:5-trichlorobenzene given to rabbits. This compound has been re-investigated and it is now clear that it is not readily metabolized.

METHODS AND MATERIALS

Melting points are corrected.

Materials. 1:3:5-Trichlorobenzene was prepared from 2:4:6-trichloroaniline and carefully purified; it had m.p. 69° (m.p. values from 61° to 64° are quoted in the literature). Pentachlorobenzene, m.p. 88° (Holleman, 1920) and hexachlorobenzene, m.p. 230° (Silberrad, 1922) were prepared and carefully purified, the samples used being judged to be free of less-chlorinated benzenes by absorption spectra (see Table 1, and tables of spectra quoted by Jondorf *et al.* 1958). *p*-Chlorophenol, m.p. 42°, and its toluene-*p*-sulphonate, m.p. 71°, and pentachlorophenol, m.p. 191°, and its benzoate, m.p. 160°, were prepared as reference compounds.

Animals. Chinchilla doe rabbits, kept throughout on a diet of 80 g. of rat cubes (diet 41; Associated London Flour Millers) and 100 ml. of water/day, were used. The chlorinated benzenes were administered either by stomach tube as aqueous suspensions or subcutaneously as 10% (w/v) solutions in arachis oil. Urine was collected daily.

Analytical methods. Glucuronic acid, ethereal sulphate and mercapturic acid outputs in urine were determined by methods already described (e.g. Jondorf *et al.* 1958). Spectrophotometric determinations were made with a Unicam spectrophotometer (SP. 500).

Estimation of chlorinated phenols. 2:4:6-Trichlorophenol in urine was determined spectrophotometrically (see Jondorf *et al.* 1955). Total pentachlorophenol in urine was determined from the difference between the light absorption of acid and alkaline solutions of steam-distillates of urines previously hydrolysed in 5N-HCl as described by Azouz *et al.* (1955) for dichlorophenols. Free pentachlorophenol was similarly estimated from steam-distillates of urine adjusted to pH 2·0 with 2N-HCl. At the wavelength used (319 mμ) the ε value for pentachlorophenol is 5400 in 0·1N-NaOH and 1200 in 0·1N-HCl. The light-absorption maxima of pentachlorophenol were at 250 and 319 mμ (ε 9700 and 5400 respectively) in 0·1N-NaOH, and at 292, 296, 300 and 304 mμ (ε 3250, 3300, 3300 and 3400 respectively) in 0·1N-HCl.

Pentachlorophenol (5 mg./100 ml.) added to normal rabbit urine (50 ml.) and hydrolysed by refluxing for 3 hr. with an equal volume of conc. HCl was recovered quantitatively (103±5%) in 0·5 vol. of distillate. Distillation from aqueous solution at pH 2·0 gave recoveries of only 75±5% in 0·5 vol. of distillate; water equal to the distillate was therefore added to the flask and the steam-distillation repeated. Three distillations in this way gave a recovery of 100±5%. Therefore a single distillation was used with hydrolysed urines, and three consecutive distillations for unhydrolysed urines.

Estimation of chlorinated benzenes. (i) In expired air. The estimation of 1:3:5-tri-, penta- and hexa-chlorobenzene in expired air was carried out spectrophotometrically as described by Azouz, Parke & Williams (1952) for other halogenated benzenes. 1:3:5-Trichlorobenzene was determined from its absorption at 280 mμ, pentachlorobenzene at 298 mμ and hexachlorobenzene at 301 mμ. Monochlorobenzene appeared to be present in some experiments and it was estimated at 264 mμ, correction being made for the absorption due to any other chlorinated benzene present.

(ii) In faeces and tissues. The estimations were carried out spectrophotometrically as described by Jondorf *et al.* (1958) for tetrachlorobenzenes. The 'pelt' included all the attached subcutaneous fat and 'depot fat' comprised all the macroscopic fat tissue excepting that of the pelt. After injection of the chlorinated benzenes, the pelt and adjacent tissues within a radius of about 2 cm. of the site of injection were separately examined.

Chromatography. The R_F values and colour reactions of the chlorophenols relevant to this work are given in Table 2.

Table 1. *Absorption spectra of penta- and hexa-chlorobenzene*

	Ethanol		*n*-Hexane	
Solvent	$\lambda_{max.}$ (mμ)	$\epsilon_{max.}$	$\lambda_{max.}$ (mμ)	$\epsilon_{max.}$
Pentachlorobenzene	288	360	261	310
	298	350	274	245
			278	250
			289	310
			298	360
Hexachlorobenzene	291	245	~280	225
	301	210	291	260
			299	230

Isolation and detection of 1:3:5-*trichlorobenzene*

In expired air. Two rabbits received orally 0·5 g./kg. of the trichlorobenzene. The expired air was drawn through ethanol (see Jondorf *et al.* 1958) and the spectrum of the ethanol was examined periodically. During the first 4 days after dosing, maxima were found only at 264, 272 and 280 mμ, which corresponded to 1:3:5-trichlorobenzene. From the fifth to the ninth days maxima also appeared at 245, 251 and 258 mμ, suggesting the presence of monochlorobenzene (see Fig. 1). With 280 mμ for trichlorobenzene and 264 mμ for monochlorobenzene (correcting for the amount of trichlorobenzene absorption at 264 mμ), it was estimated that these two rabbits eliminated, in 9 days, 8·8 and 11·9% of the dose as 1:3:5-trichlorobenzene and 1·5 and 0·6% as monochlorobenzene.

Table 2. *R_F values and colour reactions on paper of certain chlorophenols*

R_F values are for descending chromatography on Whatman no. 1 paper in solvent *A* and *B* and on Whatman no. 4 paper treated with aq. 0·2N-Na_2CO_3 in solvent *C*. Solvents: *A*, benzene–acetic acid–water (1:1:2, by vol.), run for 6 hr.; *B*, ethanol–butan-1-ol–aq. 6N-NH_3 soln.–6N-$(NH_4)_2CO_3$ (22:80:19:19, by vol.) (cf. Fewster & Hall, 1951) run for 12 hr.; *C*, *n*-hexane–di*iso*propyl ether (9:1, v/v) run for 0·5 hr. Detecting reagents were: Gibb's reagent (2% ethanolic solution of 2:6-dichloroquinonechloroimide followed by 2N-Na_2CO_3); diazotized *p*-nitraniline, followed by 2N-Na_2CO_3; diazotized sulphanilic acid, followed by 2N-Na_2CO_3; aq. 1% $FeCl_3$, followed by saturated $NaHCO_3$; 5N-HNO_3 followed by aq. 2% urea after heating the dried paper for 2 min. at 120°, followed by a solution of 0·5 g. of tetramethyl-*p*-diaminodiphenylmethane (Tetrabase) and 2 g. of citric acid in 100 ml. of water (Feigl, 1954).

	R_F values in solvent			Colour reactions				
Phenol	*A*	*B*	*C*	Gibbs's reagent	Diazotized *p*-nitraniline	Diazotized sulphanilic acid	Ferric chloride	HNO_3–Tetrabase
4-Chlorophenol	0·78	0·91	0·84	Violet	Violet	Orange	None	None
2:4:6-Trichlorophenol	0·92	—	0·10	Blue	None	None	None	None
Pentachlorophenol	0·92	0·85	0·04	None	None	None	None	Blue
4-Chlorocatechol	0·33	—	0·06	Blue	—	—	Green, neutral Blue, $NaHCO_3$	None

In faeces and tissues. 1:3:5-Trichlorobenzene (3·5 mg.), m.p. 63°, was isolated by steam-distillation of the whole of the depot fat (excluding that attached to the pelt) of one rabbit which had been dosed orally. Steam-distillates of the faeces, gut contents and the liver were shown by spectra to contain both monochlorobenzene and 1:3:5-trichlorobenzene, but depot fat, the pelt and the remaining tissues contained only 1:3:5-trichlorobenzene.

In urine. Steam-distillates of hydrolysed urines from the first 3 days after dosing showed absorption peaks at 311 and 296 mμ in 0·1 N-NaOH and at 292, 285, 280 and 275 in 0·1 N-HCl. Those at 311 mμ (in NaOH) and 292 and 285 mμ (in HCl) predominate and correspond to 2:4:6-trichlorophenol; the others were minor, and were indicative of monochlorophenols. From the fourth to the ninth days the absorption peaks due to monochlorophenols became the most predominant. Paper chromatography confirmed these findings, for in the urine excreted from 4 to 9 days after dosing both 2:4:6-trichloro- and 4-chloro-phenol were detected. No 4-chlorophenol could be detected chromatographically in the urine of the first 3 days. Paper chromatography of the residues left after steam-distillation of the hydrolysed urines of 4–9 days revealed the presence of 4-chlorocatechol in amounts equivalent to a total of about 1% of the dose.

Isolation and detection of pentachlorobenzene

In expired air. The expired air of rabbits given oral doses of 0·5 g. of pentachlorobenzene/kg. was drawn through ethanol. After 12 hr. the absorption spectrum of the ethanol showed minor peaks at 268, 272, 276 and 281 mμ. These peaks increased in intensity and reached a maximum after 60 hr. and were still detectable at 84 hr. after dosing. There was practically no light absorption at and above 290 mμ (see Fig. 2). These observations suggested that neither penta- nor tetra-chlorobenzene was appearing in the expired air. The observed peaks suggested the presence of tri- and possibly di- and mono-chlorobenzene. When the pentachlorobenzene was injected no absorption peaks appeared in the ethanol until 48 hr. after dosing, and then peaks were observed at 254, 262, 270, 276 and 282 mμ. The exhaled chlorinated benzene was assumed to be 1:3:5-trichlorobenzene and, calculating from the absorption at 272 and 280 mμ, 9 and 21% of oral doses appeared in the breath in two experiments of 3 and 4 days' duration. After injection only 1% appeared in the breath.

In faeces and tissues. Pentachlorobenzene, m.p. and mixed m.p. 88°, was readily isolated from faeces and gut contents of rabbits given pentachlorobenzene orally and from the pelt and depot fat of animals injected subcutaneously with the compound. The spectra of hexane extracts of steam-distillates of the faeces and gut contents suggested the presence of less-chlorinated benzenes.

In urine. The urine of three rabbits given a total of 4·5 g. of pentachlorobenzene was collected for 5 days and made 5 N with respect to HCl by the addition of conc. HCl. It was boiled under reflux for 3 hr. and then steam-distilled. The spectra of the distillate showed peaks at 275 and 280 mμ in 0·1 N-HCl and 292 and 297 mμ in 0·1 N-NaOH. These suggest the presence of *p*-chlorophenol and

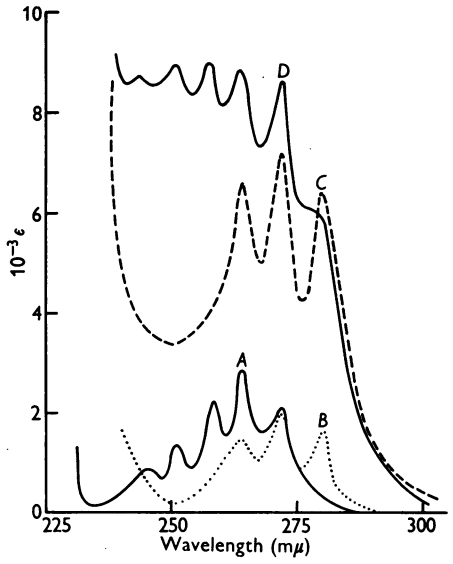

Fig. 1. Absorption spectrum in ethanol of the expired air of a rabbit after an oral dose of 1·5 g. of 1:3:5-trichlorobenzene compared with the spectra of chlorobenzene and 1:3:5-trichlorobenzene in ethanol. Ordinates for the curves of the expired air are the same but are arbitrary. Maxima (mμ): chlorobenzene (*A*), 245, 251, 258, 264, 272; 1:3:5-trichlorobenzene (*B*), —, —, —, 264, 272, 280; expired air, third day (*C*), —, —, —, 264, 272, 280; sixth day (*D*), 244, 251, 258, 264, 272, —.

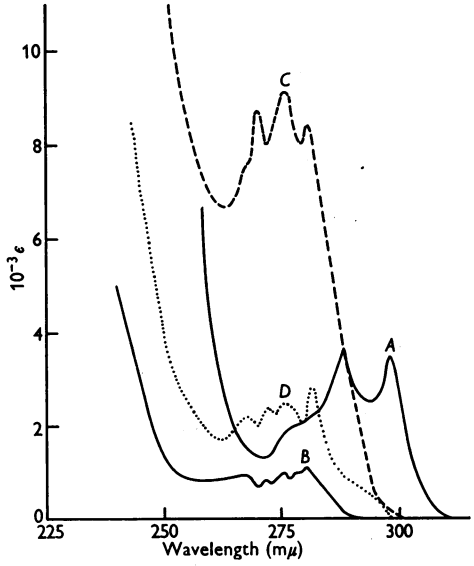

Fig. 2. Absorption spectrum in ethanol of the expired air of a rabbit after an oral dose of 1·3 g. of pentachlorobenzene compared with the spectrum of pentachlorobenzene in ethanol. Ordinates for the curves of the expired air are the same but are arbitrary. Maxima or inflexions (mμ): pentachlorobezene (*A*), —, —, —, —, —, 288, 298; expired air, first day (*B*), 268, 272, 276, 278, 281, —, —; third day (*C*), 268, 270, 276, —, 281, —, —; fourth day (*D*), 267, 272, 276, —, 282, —, —.

this was confirmed by paper chromatography. The distillates were extracted with ether, and the chlorophenols extracted were then transferred to N-NaOH. Treatment of the alkaline solution with toluene-*p*-sulphonyl chloride yielded 30 mg. of semi-solid material from which *p*-chlorophenyl toluene-*p*-sulphonate (3 mg.; m.p. and mixed m.p. 71°) was isolated. The ester on hydrolysis gave *p*-chlorophenol, which was identified chromatographically and by its spectrum in acid and alkali.

Isolation and detection of hexachlorobenzene

In expired air. Hexachlorobenzene (0·5 g./kg.) was administered orally to a rabbit and the expired air drawn through ethanol. No absorption peaks appeared in the ethanol during the 4 days of the experiment. When hexachlorobenzene was injected subcutaneously, no absorption peaks were found during the first 3 days, but on the fourth day insignificant peaks appeared at 245, 250, 255, 260, 265, 270, 278, 282 and 288 mμ.

In faeces and tissues. Hexachlorobenzene, m.p. and mixed m.p. 230°, was isolated from the faeces and gut contents of rabbits killed 5 days after being given hexachlorobenzene orally. It could also be isolated from the subcutaneous fat of rats injected with the compound. Steam-distillates of the gut contents of the rabbits fed with hexachlorobenzene were examined spectroscopically in hexane and very minor peaks were found at 278, 282 and 287 mμ in addition to the maxima corresponding to hexachlorobenzene. These might suggest the presence of 1:2:4:5-tetrachlorobenzene but the results were inconclusive. When hexachlorobenzene was injected, no chlorinated benzenes were found in the faeces after 5 days.

RESULTS AND DISCUSSION

A summary of the results obtained is given in Table 3, which shows that we have accounted for 75–85% of the chlorohydrocarbons administered. The three compounds do not undergo any extensive metabolic change *in vivo*, and it is doubtful whether hexachlorobenzene undergoes any change at all.

1:3:5-*Trichlorobenzene.* Jondorf *et al.* (1955) reported that this compound was oxidized to a small extent to 2:4:6-trichlorophenol. This has been confirmed. We have also obtained chromatographic evidence that 4-chlorophenol and 4-chlorocatechol are minor urinary metabolites. The formation of these phenols would suggest that the trichlorobenzene is partly dechlorinated to chlorobenzene, possibly by gut bacteria, and support for this was obtained on spectroscopic examination of the expired air (see Fig. 1). There was also evidence that chlorobenzene was present in the tissues. The exhalation of monochlorobenzene, however, only occurred 3–4 days after dosing with 1:3:5-trichlorobenzene. The main bulk of the administered trichlorobenzene was found unchanged in the tissues and gut contents 8–9 days after dosing (see Table 3).

Pentachlorobenzene. When given by mouth, the major portion of the dose of this compound is to be

Table 3. *Metabolism of* 1:3:5-*tri-, penta- and hexa-chlorobenzene in rabbits*

Doses were given orally unless indicated otherwise.

Compound	Dose (g./kg.)	Time after dosing (days)	Percentage of dose found in: Urine: Tri- or penta-chlorophenol	Urine: Other phenols	Faeces	Gut contents	Pelt	Depot fat	Rest of body	Expired air: Unchanged	Expired air: Other chlorohydrocarbons	Total accounted for (%)
1:3:5-Trichlorobenzene	0·5	8	3	1	13	23*	5	5	22	12	0·6	85
	0·5	9	10	4	1·5	18	5	4·5	20	8·5	1·5	74
Pentachlorobenzene	0·5	3	0·2	1	5	45	1	15	6	0	9	82
	0·5	4	0·2	1	5	31	5	9	5·5	0	21	78
	0·5†	10	0·7	1	1·5	0·5	47‡	22‡	10	0	<2	85
Hexachlorobenzene	0·4	5	0	0	6	78	<1	<1	<1	0	<0·2	84
	0·4	5	0	0	6	73	1	1	2	0	<0·2	83
	0·1†	5	0	0	<0·3	0·2	75‡	<1	<1	0	<0·5	76

* 4% as monochlorobenzene. † Injected subcutaneously. ‡ Located mainly at site of injection.

found unchanged in the gut contents after 3–4 days. Some 20 % is in the tissues generally and only about 5 % in the faeces. When injected, the main bulk of the compound is found after 10 days in the tissues near the site of injection. On oral administration, some of the hydrocarbon (about 10 %) is eliminated in a changed form in the expired air, and, from the spectrum of the expired air, this material is not unchanged pentachlorobenzene but a mixture of less-chlorinated benzenes. The suggestion that pentachlorobenzene is dechlorinated was supported by the isolation of *p*-chlorophenol in small amounts from the urine. The amounts of metabolites of pentachlorobenzene in the urine are very small, being not more than about 1 % of the dose. In four experiments pentachlorophenol was detected in the urine with certainty in only two cases. The main urinary metabolites appear to be small amounts of *p*-chlorophenol and 4-chlorocatechol, of which the former was isolated.

Hexachlorobenzene. This compound does not appear to be metabolized, and when given orally the main bulk of the dose is to be found in the gut contents after 5 days, only 6 % appearing in the faeces. There is no significant urinary or pulmonary excretion of metabolites. No attempt was made to prevent coprophagy by the animals used.

Output of conjugates after feeding with penta- and hexa-chlorobenzene. The urinary output of glucuronides, ethereal sulphates and mercapturic acids after the administration of pentachlorobenzene (0·5 g./kg.) and hexachlorobenzene (0·4 g./kg.) was determined in six animals for each compound. No significant rise in the output of these conjugates was observed, except for glucuronic acid after administering hexachlorobenzene, when an average increase in glucuronic acid output equivalent to 15 % of the dose (range 9–23 %) was observed. The increase appeared to be due to the excretion of free glucuronic acid, which was separated in the basic lead acetate fraction (Kamil, Smith & Williams, 1951) of the 2-day urine of two rabbits each dosed with 0·8 g. of hexachlorobenzene. The free glucuronic acid in this fraction was identified by colour reactions and its R_F, 0·18 in propan-1-ol–aq. NH_3 soln. (sp.gr. 0·88) (7:3, v/v) and 0 in butan-1-ol–aq. 3N-NH_3 soln. (2:3, v/v). It was concluded that penta- and hexa-chlorobenzene did not form conjugated glucuronic acids, ethereal sulphates or mercapturic acids.

SUMMARY

1. A study has been made of the fate in the rabbit of 1:3:5-tri-, penta- and hexa-chlorobenzene.

2. 1:3:5-Trichlorobenzene is not readily metabolized. The major portion of a dose of 0·5 g./kg. is unchanged in the gut contents and tissues 8 days after dosing. Some of the unchanged chlorohydrocarbon is eliminated in the faeces and expired air. A small proportion is converted into monochlorobenzene, some of which appears in the expired air (about 1 % of the dose) and as *p*-chlorophenol in the urine. Less than 10 % of the dose is excreted as 2:4:6-trichlorophenol.

3. Pentachlorobenzene is not readily metabolized. About 60 % of the oral dose (0·5 g./kg.) is found in the gut contents and tissues 3–4 days after dosing. Some 10–20 % is dechlorinated and eliminated in the expired air as less-chlorinated benzenes, and a small amount of *p*-chlorophenol was isolated from the urine, thus proving the occurrence of dechlorination. Injected pentachlorobenzene remains largely at the site of injection and was found there 10 days later. Pentachlorophenol was a very minor urinary metabolite ($< 0{\cdot}2$ % of the dose).

4. It is doubtful whether hexachlorobenzene is metabolized at all. The major portion of oral doses (0·4 g./kg.) was found in the gut 5 days after dosing and most of an injected dose (0·1 g./kg.) was found at the site of injection after 5 days.

5. Pentachlorobenzene and hexachlorobenzene gave no ethereal sulphates, glucuronides or mercapturic acids.

The expense of this work was in part defrayed by the Agricultural Research Council.

REFERENCES

Azouz, W. M., Parke, D. V. & Williams, R. T. (1952). *Biochem. J.* **50**, 702.

Azouz, W. M., Parke, D. V. & Williams, R. T. (1955). *Biochem. J.* **59**, 410.

Feigl, F. (1954). *Spot Tests*, 5th ed., vol. 2, p. 323. London: Elsevier Publishing Co. Ltd.

Fewster, M. E. & Hall, D. A. (1951). *Nature, Lond.*, **168**, 78.

Holleman, A. F. (1920). *Rec. Trav. chim. Pays-Bas*, **39**, 746.

Jondorf, W. R., Parke, D. V. & Williams, R. T. (1955). *Biochem. J.* **61**, 512.

Jondorf, W. R., Parke, D. V. & Williams, R. T. (1958). *Biochem. J.* **69**, 181.

Kamil, I. A., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 235.

Parke, D. V. & Williams, R. T. (1955). *Biochem. J.* **59**, 415.

Silberrad, O. (1922). *J. chem. Soc.* **121**, 1021.

Smith, J. N., Spencer, B. & Williams, R. T. (1950). *Biochem. J.* **47**, 284.