# Studies in Detoxication

## 42. FLUOROBENZENE. SPECTROPHOTOMETRIC DETERMINATION OF THE ELIMINATION OF UNCHANGED HALOGENOBENZENES BY RABBITS. A COMPARISON OF THE OXIDATION *IN VIVO* OF FLUOROBENZENE AND OF BENZENE

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(*Received* 14 *September* 1951)

Fluorobenzene is very similar in odour and general properties to benzene and, in fact, it resembles benzene more closely than it does the other halogenobenzenes. We shall show in the present paper that this similarity is also evident during its metabolism, for the main metabolites of fluorobenzene are excreted in amounts which are quantitatively similar to those of benzene rather than the other halogenobenzenes.

The earlier work on fluorobenzene centred on its effect on sulphur metabolism, and Coombs (1927) showed that in the dog, fluorobenzene caused a rise in the neutral sulphur output of the urine. This rise, however, was much smaller than that observed with the other monohalogenobenzenes and Coombs suggested that this could be in part due to the greater volatility of fluorobenzene. Coombs's work suggested that fluorobenzene formed a mercapturic acid *in vivo* and this was confirmed when Young & Zbarsky (1944) isolated *p*-fluorophenylmercapturic acid from the urine of rats which had received fluorobenzene orally or subcutaneously. The yields of mercapturic acid in Young & Zbarsky's experiments were about 1–2 % of the dose, which is small when compared with the amounts (25 % of the dose) which arise from chloro-, bromo- and iodo-benzenes (see for example, Spencer & Williams, 1950). Recently we have shown that, in the rabbit, benzene yields only 1–2 % of mercapturic acid (Parke & Williams, 1951) and the present work suggests the same order of mercapturic acid output in these animals when fluorobenzene is administered.

We shall also show that the halogenobenzenes can be estimated in a very simple manner by means of their light absorption in the ultraviolet.

## EXPERIMENTAL

### *Spectrophotometric determination of halogenobenzenes*

Previous methods for the estimation of halogenobenzenes include combustion and determination of the gaseous products (Thomas, Ivie, Abersold & Hendricks, 1943), the determination of the colour imparted to flames by $CuCl_2$ (Department of Scientific and Industrial Research, 1940) and nitration followed by colorimetric determination of dinitrohalogenobenzenes (Ginzberg, 1947). The spectroscopic method developed here appears to surpass all these methods in accuracy and simplicity.

*Materials.* The halogenobenzenes (British Drug Houses Ltd.) were redistilled before use and had the following boiling points (uncorr.): fluorobenzene, 83°; chlorobenzene, 130°; bromobenzene, 154°; and iodobenzene, 186°. Ethanol was of 'anhydrous for spectroscopy' grade. Spectrographic measurements were made with a Unicam Spectrophotometer, Model SP500.

*Light absorption data.* The absorption spectra of the four halogenobenzenes in ethanol are given in Fig. 1. The spectra of these compounds in hexane have been previously determined by Conrad-Bilroth (1932, 1934). Klingstedt (1933) has recorded the spectrum of iodobenzene in hexane, and Morton & Stubbs (1940) have quoted figures for chlorobenzene in ethanol.

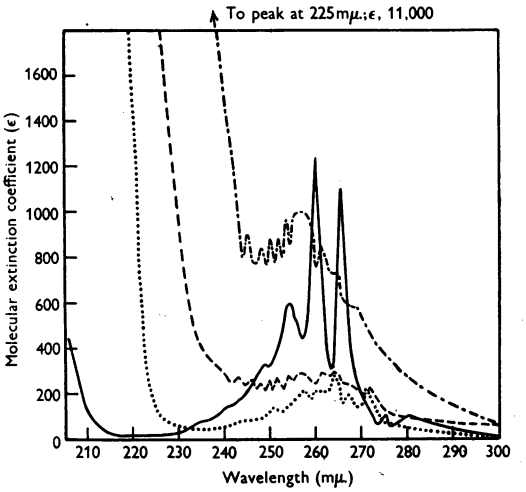


Fig. 1. Absorption spectra of fluorobenzene (—), chlorobenzene (. . . . .), bromobenzene (– – –) and iodobenzene (–·–·–) in ethanol (see also Table 1).

In the present work the selected maxima and their extinctions for the quantitative estimation of the halogenobenzenes are given in Table 1. Since the spectra of these compounds show a number of sharp peaks close together the slit width of the spectrophotometer at which the extinctions

of the maxima are determined is of great practical importance. When the molecular extinction coefficient has been determined for the pure compounds at a given slit width, the same slit width must then be used for the determination of all unknown solutions. Extinction values for the halogenobenzenes in the 260 mμ. region were found to vary appreciably with the slit width of the instrument. Data for two values of the slit width are given in Table 1.

Table 1. *Wavelengths and extinctions of absorption bands selected for the determination of halogenobenzenes in ethanol*

| | Slit width 0·2 mm. | | Slit width 0·5 mm. | |
|---|---|---|---|---|
| Compound | $\lambda_{max.}$ (mμ.) | $\epsilon_{max.}$ | $\lambda_{max.}$ (mμ.) | $\epsilon_{max.}$ |
| Fluorobenzene | 260·25 | 1,250 | 260 | 700 |
| Chlorobenzene | 264 | 280 | 263·5 | 170 |
| Bromobenzene | 264 | 300 | 263·5 | 197 |
| Iodobenzene | 225 | 11,000* | 224·5 | 10,000† |
| | 256·7 | 1,000 | 257 | 660 |

* Slit width 1·0 mm. † Slit width 1·5 mm.

The intense bands at 210–220 mμ. which occur in the spectra of fluoro-, chloro- and bromo-benzene were not suitable for accurate estimation of these compounds, since the spectrophotometer is not sufficiently reliable in this region. In the case of iodobenzene these particular bands are shifted to longer wavelengths and the peak at 225 mμ. is at a point where the instrument is reliable. The selected wavelengths of all four compounds were shown to obey the Lambert-Beer Law within the recommended scale readings ($E = 0{\cdot}3$–$0{\cdot}8$) of the spectrophotometer.

*Apparatus.* The tank used for the analysis of the expired air of rabbits was identical with that described by Parke & Williams (1950) for the study of the elimination of unchanged benzene by rabbits. The absorption train consisted of four Dreschel bottles, the first two (nearest the tank) containing a layer of glass wool on top of which was placed anhydrous $CaCl_2$ (14–20 mesh) to absorb water vapour and thus prevent ice formation. The recovery experiments quoted in Table 2 show that no halogenobenzene was lost in these bottles. The next two bottles each contained 40 ml. of 2 mm. glass beads and 25 ml. of ethanol and were kept throughout an experiment at −50° in a freezing mixture of solid $CO_2$ and acetone. All connexions were made of 'Portex' polyvinyl plastic tubing (Portland Plastics Ltd., 6 Victoria Street, London, S.W. 1), and ground-glass joints were lubricated with silicone high vacuum grease (Dow Corning Corporation, Midland, Michigan, U.S.A.).

Air was drawn through the apparatus at the rate of 50 l./hr. and the ethanol absorption bottles were renewed about every 2 hr. The halogenobenzene in the absorption bottles was estimated by pouring the ethanol and glass beads into a sintered-glass filter, washing the beads with ethanol and collecting the filtrate. The filtrate was then made up to 200 ml. at 20° with ethanol. Suitably diluted portions were then examined in the spectrophotometer in 1 cm. quartz cells.

*Recovery of halogenobenzenes.* Weighed amounts of the halogenobenzenes were introduced and spilt in the tank (see Parke & Williams, 1950). The latter was sealed and a current of air drawn through it. The amount of halogenobenzene was then estimated as above (see Table 2). The laboratory atmosphere could be simultaneously tested by means of a Y-piece placed between the absorption train and the tank. We never found any significant amounts of hydrocarbons or interfering materials in the laboratory atmosphere. The amounts of the halogenobenzenes used in these recovery experiments were those expected in our animal experiments.

### *Animal experiments*

The animals used were Chinchilla doe rabbits weighing about 3 kg. These animals were kept on a diet of 70 g. rat cubes and 150 ml. water/day. The dose of halogenobenzene was administered by stomach tube (made of polyvinyl plastic) as an emulsion in water. In some experiments with fluorobenzene, the undiluted compound was given by intraperitoneal injection. Immediately after administering the dose, the animal was placed in the tank which was sealed and connected to the absorption train. A current of air, at the rate of 50 l./hr., was then drawn through the whole apparatus until the amount of halogenobenzene eliminated was negligible; this usually took about 30 hr. or more. The absorption bottles were changed at frequent intervals and the halogenobenzene determined spectroscopically as above. A complete absorption spectrum was determined on several samples of the absorption fluid during the course of each experiment to prove that the material being determined was

Table 2. *Recoveries of halogenobenzenes from the tank estimated spectrophotometrically*

| | | | Weight recovered | | | |
|---|---|---|---|---|---|---|
| Compound | Wt. introduced into tank (g.) | Duration of aeration (hr.) | 1st bottle (g.) | 2nd bottle (g.) | Total (g.) | Total (as % of wt. introduced) |
| Fluorobenzene | 1·024* | 5 | 1·020 | 0·002 | 1·022 | 100 |
| | 1·024* | 6 | 1·015 | 0·008 | 1·023 | 100 |
| | 0·521 | 4 | 0·524 | 0·002 | 0·526 | 101 |
| | 0·516 | 5 | 0·500 | 0·000 | 0·500 | 97 |
| Chlorobenzene | 0·338 | 5 | 0·327 | 0·001 | 0·328 | 97 |
| | 0·298 | 4·5 | 0·293 | — | 0·293 | 98 |
| Bromobenzene | 0·0663 | 6·5 | 0·0655 | — | 0·0655 | 99 |
| | 0·0672 | 6·5 | 0·0658 | 0·002 | 0·0678 | 101 |
| Iodobenzene | 0·0411 | 5 | 0·0390 | 0·0001 | 0·0391 | 95 |
| | 0·0412 | 7 | 0·0403 | 0·0009 | 0·0412 | 100 |

* In these instances the fluorobenzene was recovered from a Dreschel bottle.

the halogenobenzene fed and on every occasion this was done; the spectrum obtained was that of the substance fed. Table 3 is a summary of the results and Fig. 2 shows graphically the elimination of fluorobenzene in a single experiment.

Table 3. *Elimination of unchanged halogenobenzenes in the expired air of rabbits receiving these compounds orally*

| Compound fed | Rabbit no. | Dose (g./kg.) | Duration of experiment (hr.) | Unchanged compound eliminated (as % of dose) |
|---|---|---|---|---|
| Fluorobenzene | 37 | 0·5 | 31 | 41 |
| | 37 | 0·5† | 50 | 41 |
| | 66 | 0·5 | 30 | 32 |
| | 68 | 0·5 | 32 | 53 |
| | 72 | 0·5 | 27 | 49 |
| | 66 | 0·5 | 10* | 31* |
| | 67 | 0·5 | 12* | 43* |
| | 37 | 1·0 | 30 | 73 |
| | 66 | 1·0 | 42 | 68 |
| | 68 | 1·0 | 32 | 57 |
| | 72 | 1·0 | 48 | 59 |
| Chlorobenzene | 37 | 0·5 | 30 | 32 |
| | 68 | 0·5 | 30 | 24 |
| | 71 | 0·5 | 30 | 25 |
| Bromobenzene | 71 | 0·5 | 26 | 5·5 |
| | 72 | 0·5 | 26 | 7·0 |
| Iodobenzene | 37 | 0·5 | 30 | 3·8 |
| | 72 | 0·5 | 30 | 2·7 |

* Incomplete experiments.
† Injected intraperitoneally.

*Experiments on the oxidation of fluorobenzene*

Qualitative tests on the urine from rabbits fed with fluorobenzene showed the presence of fluorophenols, and the extent of their formation was assessed by determinations of ethereal sulphate and glucuronic acid in fluorobenzene urine.

*Glucuronic acid* was determined by the method of Hanson, Mills & Williams (1944) using pure D-glucurone as standard. The addition of 0·1 mg. of $K_2S_2O_8$ in 0·1 ml. water to each tube prior to immersion in the boiling-water bath was found to improve the method.

*Ethereal sulphate* was determined by the turbidimetric method of Sperber (1948) with the modification that the standard curve was made with $(NH_4)_2SO_4$, not in aqueous solution, but in diluted (1:4) rabbit urine. The standard curve in rabbit urine was not the same as in aqueous solution. Using this method, the recovery of potassium *p*-chlorophenylsulphate (0·5–1·5 mg. in 2 ml. urine) was 98–105%.

*Mercapturic acid* was determined iodometrically (Stekol, 1936; cf. Parke & Williams, 1951). We have not proved, however, that the urine of rabbits receiving fluorobenzene contains a mercapturic acid and therefore our figures for mercapturic acid are only given tentatively, although no other metabolite has been found which consumes iodine under the conditions of the estimation.

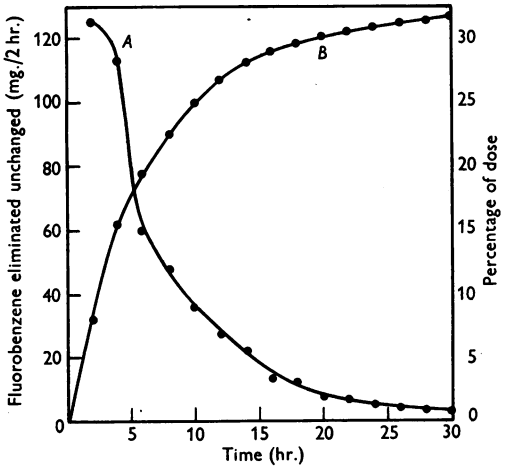


Fig. 2. The elimination of unchanged fluorobenzene in the expired air of rabbit no. 66 which had received 1·536 g. of fluorobenzene orally. *A*, curve showing the amount of fluorobenzene in mg. eliminated unchanged in each 2 hr. period after dosing. *B*, curve showing the total amount of fluorobenzene eliminated as a percentage of the dose.

The results of the above determinations are given in Table 4. This table shows that the total *O*-conjugation of fluorobenzene is about 30% of the dose. With rabbits nos. 32, 39 and 55 some observations were made on the effect of the time of giving normal food on the glucuronic acid and ethereal sulphate conjugations (cf. Bray & White, 1951). In Exps. 1, 3 and 5 the animals were allowed access to their food immediately after dosing with fluorobenzene, whereas

Table 4. *Effect of fluorobenzene on the glucuronic acid, ethereal sulphate and mercapturic acid output of rabbits*

| Exp. no. | Rabbit no. | Dose (g./kg.) | Extra glucuronic acid, *G* (as % of dose) | Extra ethereal sulphate, *E* (as % of dose) | *E* + *G* | Apparent 'mercapturic acid'* (as % of dose) |
|---|---|---|---|---|---|---|
| 1 | 55 | 0·8 | 6·7 | 25·5 | 32·2 | 1·5 |
| 2 | 55 | 0·8 | 12·6 | 22·9 | 35·5 | 1·9 |
| 3 | 39 | 0·8 | 6·2 | 21·1 | 27·3 | 1·5 |
| 4 | 39 | 0·8 | 10·8 | 17·8 | 28·6 | 1·8 |
| 5 | 32 | 0·7 | 4·8 | 26·8 | 31·6 | 1·4 |
| 6 | 32 | 0·7 | 10·9 | 24·1 | 35·0 | 1·6 |
| 7 | 51 | 0·5 | 12·0 | 14·8 | 26·8 | — |
| 8 | 53 | 0·5 | 13·7 | 18·7 | 32·4 | — |
| 9 | 68 | 0·5 | 13·2 | 18·5 | 31·7 | — |

* By iodometric titration (see above).

in Exps. 2, 4 and 6 the animals were given their food 4 hr. after dosing. In these experiments the time of giving food had no effect on the total conjugation, but in those cases where food was allowed immediately after dosing with fluorobenzene there was an increase in ethereal sulphate conjugation and a decrease in glucuronic acid conjugation. In Exps. 7, 8 and 9, the time of giving food was not recorded. Much, of course, depends in this type of experiment upon whether or not an animal has lost its appetite temporarily after dosing with foreign compounds. With fluorobenzene the animals did not lose their appetite as they did after dosing with benzene.

*Experiments on the oxidation of benzene*

The ethereal sulphate and glucuronic acid outputs of rabbits receiving benzene have been determined by Porteous & Williams (1949). In order to compare fluorobenzene with benzene under exactly the same conditions, the glucuronic acid and ethereal sulphate outputs of rabbits receiving benzene have again been determined. The methods used were those described in the preceding section. The results are given in Table 5.

Table 5. *Effect of an oral dose of benzene on the glucuronic acid and ethereal sulphate output of rabbits*

(Dose, 0·5 g./kg.)

| Rabbit no. | Extra glucuronic acid, *G* (as % of dose) | Extra ethereal sulphate, *E* (as % of dose) | *G*+*E* |
|---|---|---|---|
| 51 | 8·8 | 17·1 | 25·9 |
| 53 | 8·7 | 22·3 | 31·0 |
| 68 | 20·4 | 32·0 | 52·4 |
| 75 | 12·8 | 15·3 | 28·1 |
| 82 | 10·4 | 30·1 | 40·5 |
| 87 | 6·4 | 31·4 | 37·8 |

In these experiments the animals were allowed access to their food immediately after dosing with benzene, but in many cases the animals had temporarily lost their appetite.

## DISCUSSION

Our results show that a considerable amount of fluorobenzene when fed by mouth is eliminated in the expired air in the unchanged state, for at dose levels of 0·5 and 1·0 g./kg., 44 and 64 % of the dose respectively are so eliminated. Fluorobenzene is thus similar to benzene in this respect (see Parke & Williams, 1950). The other halogenobenzenes are also eliminated to some extent in the expired air. Chlorobenzene, despite its much higher boiling point and much lower vapour pressure, is eliminated unchanged to the extent of 25–30 %. Even the high-boiling bromo- and iodo-benzenes are also eliminated to a small but appreciable extent in the expired air (see Tables 3 and 6). In fact, more of the chloro-, bromo- and iodo-benzenes is eliminated than would be expected from their high boiling points and low vapour pressures. This suggests the possibility that they are eliminated by a process analogous to steam distillation which depends on molecular weight as well as vapour pressure. It is to be noted that the elimination of the halogenobenzenes at a dose level of 0·5 g./kg. takes about 30–40 hr. In one instance fluorobenzene was injected and some 50 hr. was needed for the elimination of the unchanged material (Table 3).

Some 30 % of fluorobenzene is also oxidized *in vivo* and about one-third of the oxidized fluorobenzene appears as glucuronide and two-thirds as ethereal sulphates (Table 4). Preliminary experiments have shown that the oxidized forms are fluorophenols. The quantitative aspects of fluorobenzene oxidation are very similar to those of benzene (compare Tables 4 and 5 and see summary in Table 7). The scatter in the figures for benzene is larger than that found in those for fluorobenzene. Porteous & Williams (1949) also reported a considerable scatter in their figures for the glucuronic acid and ethereal sulphate output of rabbits receiving benzene.

In Table 7 the quantitative aspects of the metabolism of benzene and the halogenobenzenes have been summarized. Although all the figures given in this table are not strictly comparable because the sulphur and oxygen conjugations of the chloro-, bromo- and iodo-benzenes are for lower doses than for the other metabolites, they show that fluorobenzene is metabolically similar to benzene rather than to the halogenobenzenes. The main difference between benzene and fluorobenzene on the one hand, and chloro-, bromo- and iodo-benzene on the other, is in the extent of mercapturic acid excretion which is very low for the first two compounds even when the amount excreted unchanged is allowed for, but accounts for nearly 25 % of the last three compounds. The reason for this will not be clear until

Table 6. *Boiling points, vapour pressures and the elimination by rabbits of halogenobenzenes*

| Compound | B.p. | Vapour pressure at 30°* (mm.) | Mol.wt. | Percentage of dose eliminated by rabbits in expired air |
|---|---|---|---|---|
| Benzene | 80 | 117·5 | 78 | 39 |
| Fluorobenzene | 85 | 96·6 | 96 | 44 |
| Chlorobenzene | 132 | 15·5 | 112·5 | 27 |
| Bromobenzene | 155–6 | 5·7 | 157 | 6·3 |
| Iodobenzene | 189 | 1·5 | 204 | 3·3 |

* From Young (1910).

Table 7. *Summary of the major metabolites of benzene and the halogenobenzenes*

(Values are expressed as % of dose.)

| Compound | Glucuronide | Ethereal sulphate | Mercapturic acid | Unchanged | Total |
|---|---|---|---|---|---|
| Benzene | 11 | 25 | 1 | 39* | 76 |
| Fluorobenzene | 10 | 21 | 1·6 | 44 | 77 |
| Chlorobenzene | 25† | 27† | 20† | 27 | 99 |
| Bromobenzene | 40† | 37† | 21† | 6 | 104 |
| Iodobenzene | 31† | 30† | 23† | 3 | 87 |

* Parke & Williams (1950). † Values taken from Spencer & Williams (1950); doses low.

some explanation of the high conjugation of chloro-, bromo- and iodo-benzene with cysteine has been found. Experiments directed towards finding this explanation are now in progress.

Since fluorobenzene behaves metabolically like benzene it might be expected to give rise to a muconic acid as does benzene. Theoretically fluorobenzene could give rise to an α- or a β-monofluoromuconic acid (COOH.CH:CH.CH:CF.COOH and COOH.CH:CH.CF:CH.COOH). Both these acids on β-oxidation should yield fluoroacetic acid which is a highly toxic substance (McCombie & Saunders, 1946; Saunders, 1947; Buffa & Peters, 1949). Fluorobenzene, however, is relatively non-toxic, and on these grounds it seems unlikely that it gives rise to fluoroacetic acid *in vivo*. So far we have found no evidence for the formation of a fluoromuconic acid from fluorobenzene in rabbits. We have shown that 0·6 % of benzene is converted to muconic acid in the rabbit (Parke & Williams, 1951). If fluorobenzene were converted to fluoromuconic acid to the same extent and fluoromuconic acid were completely metabolized to fluoroacetic acid, then a dose of 0·5 g. of fluorobenzene/kg. would give rise to 2·3 mg. of fluoroacetic acid/kg. On feeding muconic acid, however, to rabbits, only 40 % is metabolized (Parke and Williams, unpublished observations). It is possible, therefore, that 0·5 g. of fluorobenzene/kg. could give rise to 0·92 mg. of fluoroacetic acid/kg.; this is nearly four times the L.D.$_{50}$ of fluoroacetic acid which is 0·25 mg./kg. (Saunders, 1947). However, until fluoromuconic acid or some other fluorine-containing open-chain compound has been isolated from fluorobenzene urine further speculation regarding the formation of fluoroacetic acid seems unprofitable.

## SUMMARY

1. A study has been made of the elimination, in the expired air, of unchanged fluoro-, chloro-, bromo- and iodo-benzene after oral administration of these substances to rabbits.

2. A spectrophotometric method for the determination of these halogenobenzenes in expired air has been described. The ultraviolet absorption spectra of these compounds in ethanol are recorded and discussed.

3. Of doses of 0·5 and 1 g. of fluorobenzene/kg., 44 and 64 % respectively are eliminated unchanged in the expired air. At a dose level of 0·5 g./kg. 27 % of chloro-, 6 % of bromo- and 3 % of iodo-benzene is excreted unchanged in the expired air. These results are discussed.

4. The elimination of fluorobenzene in the urine as ethereal sulphates, glucuronides and mercapturic acid has been studied quantitatively and the results suggest that it behaves metabolically like benzene rather than the other halogenobenzenes. Whereas chloro-, bromo- and iodo-benzenes yield large amounts (about 25 %) of mercapturic acid in the urine, benzene and fluorobenzene yield only small amounts (about 1–2 %).

5. Fluorobenzene in single doses up to 1 g./kg. is not highly toxic to rabbits.

The expenses of this work were in part defrayed by a grant from the Medical Research Council.

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