Structure-dependent, selective localization of chlorinated xenobiotics in the cerebellum and other brain structures[1]

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Summary. Remarkable regional distribution patterns of some chlorinated xenobiotics in the CNS are described.

Since its introduction in the 1950s the technique of whole-body autoradiography[2] has been used to study the distribution of a large number of chemicals in the animal body[3]. The uptake of radiolabelled compounds in the brain has been a rare finding, observed most frequently after the administration of drugs active in the CNS. Retention times in the nervous tissue of more than a few hours have been exceptional.

The extension of the whole-body autoradiographic technique to the study of distribution patterns of environmental pollutants and industrial chemicals has recently revealed a highly selective and structure-dependent localization of some chlorinated xenobiotics (fig. 1) in the cerebellum and cerebrum. The retention times have occasionally been impressively long.

The organic solvent chloroform (fig. 1, **I**) has thus been shown to accumulate immediately after a short period of inhalation in the cerebellar cortex of the mouse (fig. 2, **Ia**), and it still remained there 48 h after exposure. The compound was visualized by a low-temperature technique as described earlier[4]. No radioactivity was visible in the cerebellar cortex on autoradiograms made from dried and evaporated sections (fig. 2, **Ib**). The structure-dependency of the cerebellar localization was obvious upon comparison with the CNS distribution patterns of the less chlorinated solvent methylene chloride (fig. 1, **II**) and the more chlorinated solvent carbon tetrachloride (fig. 1, **III**), both shown by low-temperature autoradiography not to be present in the cerebellum (figs 2, **II**; 2, **III**).

The second observation concerns derivatives of the environmental pollutant hexachlorobenzene[5] (fig. 1, **VI**). Hexachlorobenzene is metabolized in vivo to methylthiopentachlorobenzene[6] (fig. 1, **V**), which after i.v. injection was found to be evenly distributed in the mouse brain (fig. 2, **V**). Methylsulphonylpentachlorobenzene, a close structural analogue of methylthiopentachlorobenzene identified as an oxidation product in vitro[7] (fig. 1, **IV**), was, however, found to have a strong affinity for the cerebellar and cerebral cortex (fig. 2, **IV**).

An i.v. dose of the insecticide γ-hexachlorocyclohexane (fig. 1, **IX**) to the mouse was also found to be evenly distributed in the brain (fig. 2, **IX**). The same even distribution pattern was established for the β-isomer of hexachlorocyclohexane (Figs 1, **VIII**; 2, **VIII**). A highly selective and long term retention (up to 4 days) in the white matter of the cerebellum and cerebrum could, however, be observed after an i.v. injection of the α-isomer of hexachlorocyclohexane (figs 1, **VII**; 2, **VII**). This localization has also been found in the rat[8]. The overall distribution patterns of the 3 hexachlorocyclohexane isomers were the same as those previously described in mice[9].

These chlorinated xenobiotics may all be assumed to possess the ability to penetrate the blood-brain barrier by virtue of their high lipid solubility. Whole-body autoradiography has, in addition to a demonstration of this penetration, also revealed remarkable localization phenomena for some of the xenobiotics in specific anatomic loci of the brain. The reasons for the highly selective affinity of these chlorinated xenobiotics for such loci remain obscure. However, the pronounced structure-dependency and the long retention times observed would seem to indicate that interactions with binding sites of high selectivity in the CNS have occurred. Simple physicochemical or physiological conditions, such as regional variation in lipid content or blood flow, can probably be excluded as factors governing the intracerebellar and intracerebral distribution of chloroform, methylsulphonylpentachlorobenzene and α-hexachlorocyclohexane in favour of unknown regional brain binding characteristics.

The presence of chlorine in the compounds found to localize in specific brain structures raises the question of whether chlorine or other halogen substituents are prerequisite for cerebellar or cerebral affinity. However, a non-halogenated compound, ethylene oxide, has also shown a conspicuous affinity for the cerebellar cortex[10] (figs 1, **X**; 2, **X**). Since no data seem to exist on the distribution of other epoxides in the brain. the structure-dependency of this localization cannot be assessed.

The toxicological significance of the observed high concentrations of chloroform, methylsulphonylpentachlorobenzene, α-hexachlorocyclohexane and ethylene oxide in different areas of the CNS remains to be investigated. However, in the case of chloroform, the observed clinical picture of chloroform poisoning with signs of ataxia, dysarthria and

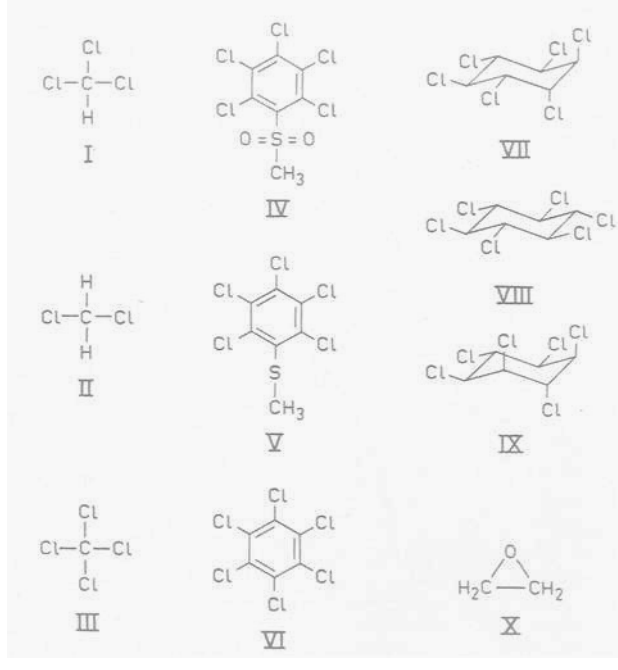

Figure 1. Structural formulae of the compounds studied: chloroform (**I**), methylene chloride (**II**), carbon tetrachloride (**III**), methylsulphonylpentachlorobenzene (**IV**), methylthiopentachlorobenzene (**V**), hexachlorobenzene (**VI**), α-hexachlorocyclohexane (**VII**), β-hexachlorocyclohexane (**VIII**), γ-hexachlorocyclohexane (**IX**) and ethylene oxide (**X**).

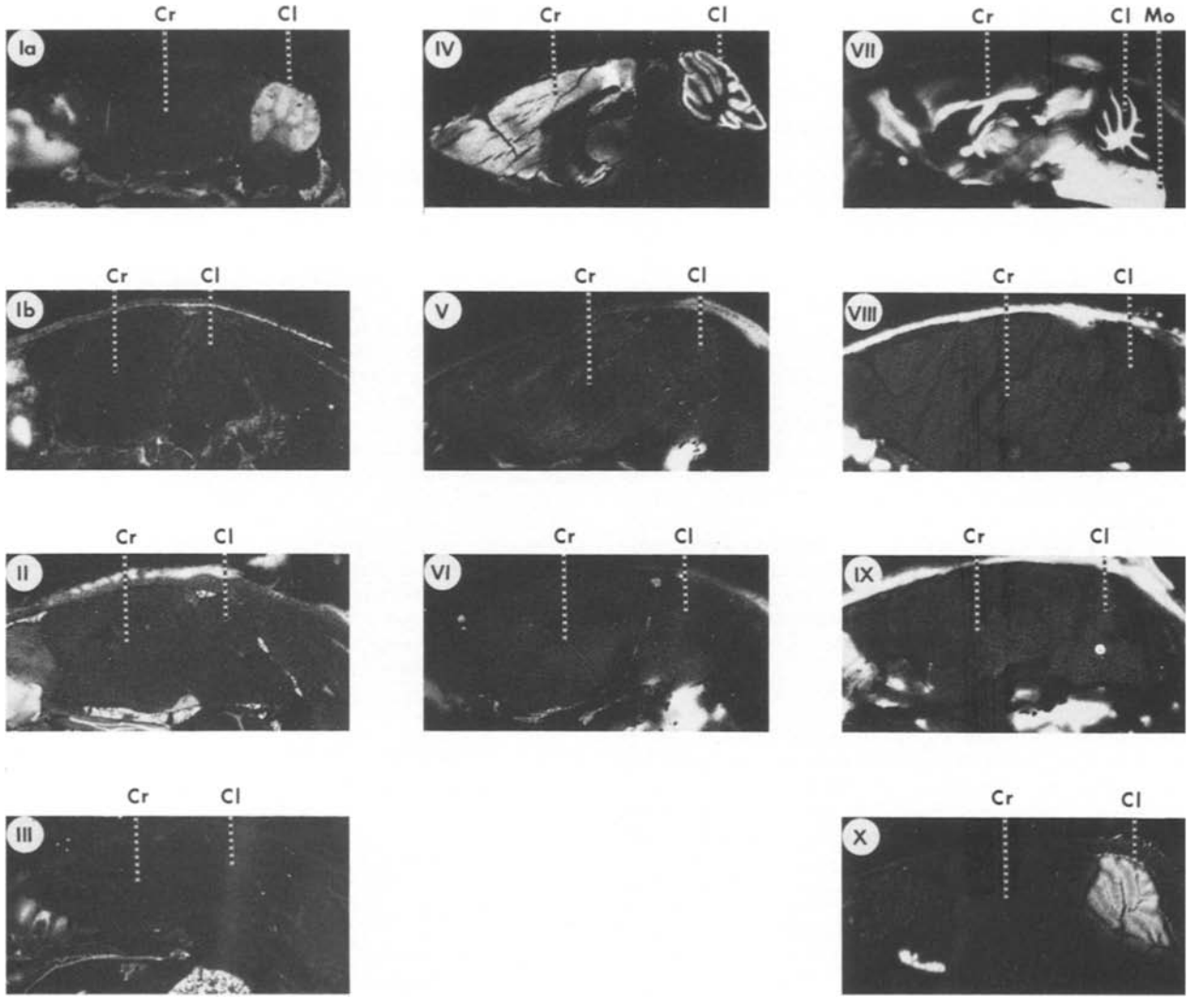

Figure 2. Details of whole-body autoradiograms of mice showing the distribution patterns of compounds **I**–**X** in the cerebrum (Cr), cerebellum (Cl) and medulla oblongata (Mo). All compounds were labelled with carbon-14. White areas represent high levels of radioactivity. Compounds **I**–**III** were administered by inhalation, compounds **IV**–**X** by i.v. injection. **Ia**: chloroform, survival time (st) 24 h, 2.3 mmole/kg, low-temperature autoradiography; **Ib**: chloroform, st 24 h, 2.3 mmole/kg; **II**: methylene chloride, st 24 h, 5.4 mmole/kg, low-temperature autoradiography; **III**: carbon tetrachloride, st 24 h, 1.7 mmole/kg, low-temperature autoradiography; **IV**: methylsulphonylpentachlorobenzene, st 1 h, 14.5 µmole/kg; **V**: methylthiopentachlorobenzene, st 1 h, 14.5 µmole/kg; **VI**: hexachlorobenzene, st 1 h, 14.5 µmole/kg; **VII**: α-hexachlorocyclohexane, st 4 h, 4.6 µmole/kg; **VIII**: β-hexachlorocyclohexane, st 4 h, 7.5 µmole/kg; **IX**: γ-hexachlorocyclohexane, st 4 h, 5.2 µmole/kg; **X**: ethylene oxide, st 24 h, 30.3 µmole/kg.

tremor[11,12] may well correspond to the high uptake of the solvent in the cerebellar cortex.

Despite the difficulties of interpretation of these rare localization phenomena, and lack of evidence of toxic effects, we have considered that these autoradiographic findings warrant attention, and further research should be encouraged on the interactions between structural elements of the brain and xenobiotics, as well as on the potential toxic response to these interactions.

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