

**REDUCTIVE DECHLORINATION OF CERTAIN
CHLORINATED ORGANIC COMPOUNDS BY REDUCED
HEMATIN COMPARED WITH THEIR BEHAVIOUR
IN THE ENVIRONMENT**

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NWRI Contribution No. 90-27

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MANAGEMENT PERSPECTIVE

Large quantities of certain chlorinated organic compounds were released into the environment earlier in the present century. These include pesticides such as DDT, lindane, mirex, aldrin, and related compounds, as well as industrial products and by-products. The manufacture and use of most of these has been stopped or greatly curtailed in recent years, but significant quantities of them or their transformation products can still be detected in environmental samples in almost all parts of the world. Consequently the ultimate fate of these materials remains a matter of concern.

These compounds are generally very resistant to oxidative degradation, which led to the fear that they might remain in the environment almost indefinitely. More recently however it has been found that many of them readily undergo a reductive reaction which removes chlorine atoms, yielding products which are much more susceptible to further degradation than were the original materials. This reaction occurs under anoxic conditions such as occur in sediments. This paper is a further contribution to knowledge of this process. It has been found that a number of otherwise recalcitrant compounds are readily dechlorinated in the presence of hematin, a substance which is widely distributed in living organisms.

It seems likely therefore that many or most organochlorine compounds in the environment will eventually be broken down by natural processes, so that there is no need to take special measures for their removal.

PERSPECTIVE GESTION

Certains composés organochlorés ont été rejetés en quantité dans l'environnement au cours du siècle. Il s'agit notamment du DDT, du lindane, du mirex, de l'aldrine et autres composés apparentés, ainsi que de produits et de sous-produits industriels. Dans la plupart des cas, on en a cessé ou réduit considérablement la production et l'utilisation ces dernières années, mais presque partout dans le monde, on en détecte encore, à l'état original ou sous forme de produits de transformation, en quantités notables dans les échantillons d'origine environnementale. Il y a donc encore lieu de s'inquiéter de ce qu'il advient finalement de ces substances.

Comme ces composés sont généralement très résistants à la dégradation par oxydation, on a craint qu'ils restent presque indéfiniment dans l'environnement. Par la suite toutefois, on a découvert que bon nombre d'entre eux subissent rapidement une réaction de réduction dans laquelle ils perdent leurs atomes de chlore et forment des produits qui se prêtent beaucoup mieux à une dégradation plus avancée que le composé original. Cette réaction s'opère en l'absence d'oxygène, condition qui existe dans les sédiments. L'article qui suit vient ajouter aux connaissances recueillies jusqu'ici sur ce processus. On a découvert qu'un certain nombre de composés ne se prêtant pas à la déchloration par les processus habituels sont rapidement déchlorés en présence

d'hématine, une substance qu'on trouve chez un grand nombre d'organismes vivants.

Puisqu'il semble très probable qu'un bon nombre, sinon la plupart, des composés organochlorés présents dans l'environnement finiront par être décomposés par des processus naturels, il n'est pas nécessaire de prendre des mesures d'élimination spéciales.

RÉSUMÉ

On a étudié un certain nombre de composés organochlorés d'intérêt environnemental pour en déterminer la réactivité avec l'hématine réduite. On a constaté que quelle que soit la classe à laquelle les substance étudiées appartiennent, leur réactivité est en assez bonne corrélation avec leur persistance dans l'environnement. Le DDT et le DDD ont réagi, mais non le DDE. Le lindane, le moins persistant des isomères du BHC s'est révélé le plus réactif. L'aldrine et l'heptachlore, moins persistants que les époxydes qu'ils forment, ont été plus réactifs. On a trouvé du chlordané parmi les produits de déchloration de l'heptachlore. Le mirex fait exception : il est assez réactif, bien qu'il soit très persistant. On a trouvé deux produits de déchloration, l'un résultant de la perte d'un atome de chlore, l'autre de la perte de deux atomes. L'hexachlorobutadiène a réagi lentement, l'octachlorostyrène, très lentement, formant de l'heptachlorostyrène. Les composés purement aromatiques n'ont pas réagi.

REDUCTIVE DECHLORINATION OF CERTAIN CHLORINATED ORGANIC COMPOUNDS
BY REDUCED HEMATIN COMPARED WITH THEIR BEHAVIOUR IN THE ENVIRONMENT

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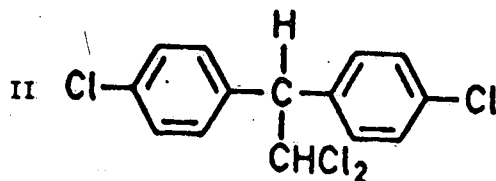
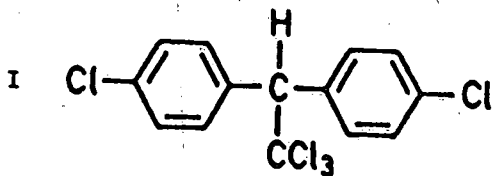
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ABSTRACT

A number of chlorinated organic compounds of environmental concern were tested for their ability to react with reduced hematin. Within any class of substances the reactivity correlated fairly well with environmental persistence. DDT and DDD reacted but DDE did not. The least persistent BHC isomer, lindane, was the most reactive. Aldrin and heptachlor, which are less persistent than their epoxides, proved more reactive. Chlordane was detected as a dechlorination product of heptachlor. Mirex proved to be an exception, being fairly reactive in spite of its great persistence. Two dechlorination products were detected, representing the loss of one and two chlorine atoms, respectively. Hexachlorobutadiene reacted slowly, and octachlorostyrene reacted very slowly, yielding heptachlorostyrene. Purely aromatic compounds did not react.

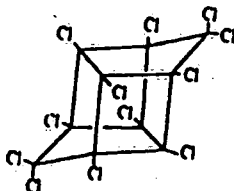
INTRODUCTION

A number of important environmental contaminants are relatively resistant to oxidative transformation under natural conditions but undergo reductive change quite readily (see, e.g., Hill & McCarty 1967; Sethunathan 1973; Macaladay et al. 1986; Tsukano 1986; Baxter 1990). One of the most important reactions of this type is reductive dechlorination. Thus it has been known for some time that 1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT,I) is reductively dechlorinated to 2,2-bis(4-chlorophenyl) 1,1-dichloroethane (DDD,II) under a variety of conditions (Kallman & Andrews 1963; Barker & Morrison 1964; Wedemeyer 1966; Ecobichon & Saschenbrecker 1967). More recently it has been found that commonly used solvents such as trichloro- and tetrachloroethylene undergo reductive dechlorination in groundwater (Parsons et al. 1984) and soil (Kleopfer et al. 1985).



A possible mechanism for these processes is reaction with reduced iron porphyrins such as hematin. Reactions of this type have been shown to lead to the rapid reductive dehalogenation of a variety of alkyl halides. DDT is converted to DDD (Castro 1964; Wade & Castro 1973a,b; Zoro et al. 1974) which may react further to form a variety of other products (Marei et al. 1978; Quirke et al. 1979). Chlorinated methanes and ethanes are converted to lower chlorinated homologues (Klečka & Gonsior 1984). Mirex (III), which is one of the most persistent of environmental contaminants, reacts readily with reduced hematin to yield a number of products (Holmstead 1976). Several of the components of toxaphene, which is a mixture of scores of components, mostly chlorinated norbornanes, are also dechlorinated by reduced hematin (Khalifa et al. 1976).

III



MATERIALS AND METHODS

Aldrin, BHC, dieldrin, heptachlor, lindane and mirex were obtained from Supelco Corp. Heptachlor epoxide was obtained from Chem Service, Inc. Octachlorostyrene was kindly provided by Dr. Barry Oliver, who received it as a gift from Dow Chemical Co. Arochlors were obtained from Monsanto. Other reagents were obtained from regular chemical supply houses.

The experimental procedure was based on that of Zoro et al. (1974). Experiments were performed by mixing substrate (2 mg/mL in acetone), potassium phosphate buffer (0.1 M, pH 7.5), Tween 80 (25 mg/mL in ethanol), hematin (0.05, 0.5 or 5.0 mg/L in 0.1 M aqueous sodium carbonate) and distilled water in the proportions of 1:1:2:2:4, in 16 x 150 mm screw-cap culture tubes. 4.0 mL portions were withdrawn for analysis then approximately 50 mg solid sodium dithionite was added. The tubes were capped, gently shaken until the contents assumed a uniform pink colour, indicating that the hematin was completely reduced, and incubated in a water bath at 25°C. Samples were withdrawn for analysis at appropriate intervals.

Control experiments were carried out to establish that both hematin and dithionite were required for dechlorination to occur.

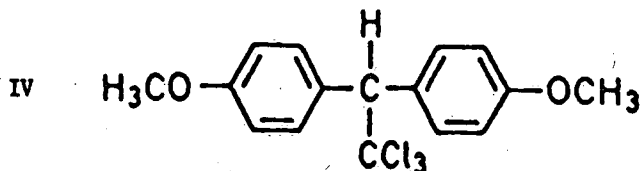
Analyses were performed by extracting with 2.0 mL ethyl acetate and injecting 1.0 µL portions onto a 30m DB-5 fused silica capillary column in a Varian Vista 6000 gas chromatograph equipped with a flame ionization detector and programmed from 40 to 260°C.

Electron impact mass spectra were obtained using a Ribier-Nermag mass spectrometer and a Carlo-Erba 4160 gas chromatograph with a 30m DB-5 fused silica column, programmed from 70 to 285°C.

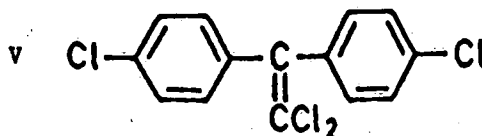
RESULTS AND DISCUSSION

DDT and related compounds. DDT reacted rapidly at the highest hematin concentration, being totally transformed within 24 hours. Part could be accounted for as DDD. Over longer periods, DDD also disappeared. No products could be detected by the analytical method used.

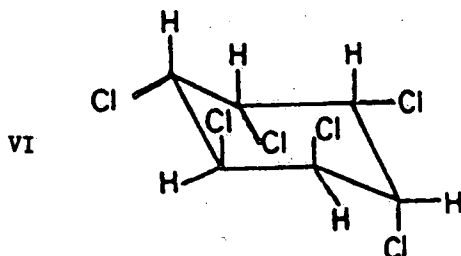
Methoxychlor (IV), an analogue of DDT, disappeared at about the same rate as DDT under the same conditions yielding no detectable products.



2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE,V), a metabolite of DDT produced by many organisms (Brooks 1974b) which is usually the predominant DDT derivative in environmental samples, showed no evidence of reaction within two weeks.



Benzene hexachloride. Benzene hexachloride (BHC) is a commercial mixture of isomers of 1,2,3,4,5,6-hexachlorocyclohexane used as an insecticide. Only one component, the γ -isomer (VI), is significantly toxic to insects. This is sometimes used in a pure form under the name of lindane.



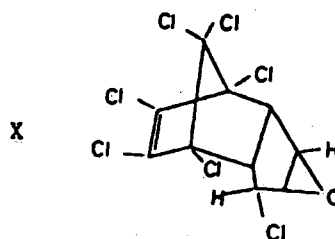
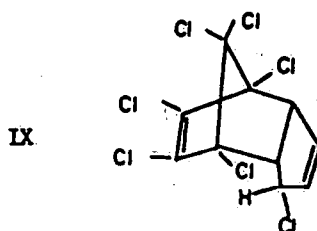
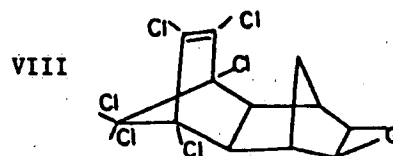
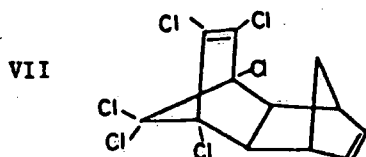
Lindane was the most reactive of all the substrates tested. Even at the lowest hematin concentration tested, 0.01 mg/mL, it was completely converted into undetectable products within 24 hours.

The sample of BHC used was found to consist primarily of three isomers. One of these had a retention time identical with that of lindane and was presumably the γ -isomer. Another had a slightly shorter retention time and was therefore probably the α -isomer (MacRae et al. 1967). The third had a longer retention time and was probably either the β - or the δ -isomer. At a hematin concentration of 0.01 mg/mL, the intermediate peak behaved like lindane and disappeared within 24 hours. The other peaks showed little change during this period and even after a week were still about one-third of their original values.

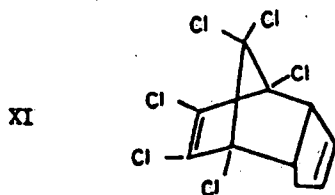
These observations are consistent with the general reactivity of these compounds, which differs greatly from one isomer to another (see, e.g., March 1985). They are also

consistent with their reported behaviour in flooded soils, where the γ -isomer has been shown to disappear significantly more rapidly than the others (MacRae et al. 1967).

Bridged polycyclic chlorinated hydrocarbons. These compounds are synthesized by a Diels-Alder reaction between hexachlorocyclopentadiene and a suitable dienophile. Several were formerly widely used as insecticides. These include mirex (III), aldrin (VII), its epoxide, dieldrin (VIII) and heptachlor (IX). Heptachlor epoxide (X) is a metabolite of heptachlor produced by a variety of organisms (Brooks 1974b).



Of these, heptachlor proved to be the most reactive. At a hematin concentration of 0.01 mg/mL it disappeared almost completely within 24 hours. In some experiments a product could be detected which represented a few percent of the original heptachlor. This gave a mass spectrum with a base peak at $m/z = 66$ ($C_5H_6^+$). This matches the reported spectrum of chlordene (XI)



which decomposes by a retro-Diels-Alder reaction in which hexachloropentadiene is eliminated as the neutral fragment and the cyclopentadiene ion is formed (Damico et al. 1968).

Heptachlor is commonly manufactured by the chlorination of chlordene (Brooks 1974a) so it was suspected that the chlordene detected might have been a contaminant. However, no trace of chlordene could be detected in the heptachlor sample before reaction, so it appears that it was a true dechlorination product.

Heptachlor epoxide underwent little or no reaction under conditions where heptachlor rapidly disappeared.

Aldrin was considerably less reactive than heptachlor. At a hematin concentration of 1.0 mg/mL it decreased by one-half to two-thirds in 24 hours, and at a concentration of

0.1 mg/mL it decreased by about one quarter. Dieldrin was less reactive, showing little change in 24 hours at a hematin concentration of 0.1 mg/mL. No reaction products could be detected with either aldrin or dieldrin.

The rates of reaction of these four compounds appear to be approximately in the reverse order of their environmental persistence.

Mirex proved remarkably reactive, as reported previously (Holmstead 1976). It appeared to react about as rapidly as aldrin. The principal product detected had a mass spectrum with a five-chlorine cluster at $m/z = 236$ ($C_5HCl_5^+$). The spectrum of mirex displays a large peak at $m/z = 270^+$ due to the hexachloropentadiene ion produced by a retro-Diels-Alder reaction (Uk et al. 1972). By analogy it seems likely that the peak at 236 represents pentachloropentadiene ion produced in the same way, and that the material was a deschloromirex, $C_{10}HCl_{11}$. A very small quantity of a second product was detected. This had a spectrum with a four-chlorine cluster at $m/z = 202$ ($C_5HCl_4^+$). By the same reasoning this would appear to be a tetrachloropentadiene ion, and the original material a bis-deschloromirex, $C_{10}H_2Cl_{10}$.

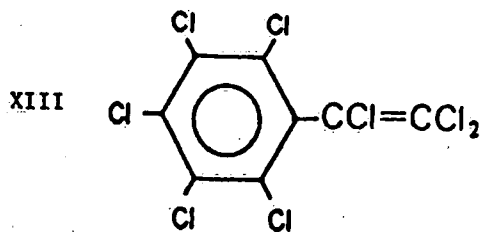
The facility with which mirex undergoes reductive dechlorination is surprising in the light of its extreme persistence in the environment. Perhaps its very low water solubility (Smith et al. 1978; Suntio et al. 1988) is responsible for its apparent lack of reactivity in nature.

Other compounds. Hexachlorobutadiene (HCBD, XII) has been detected in sediments in industrial areas in several parts of the world. It is probably produced as a by-product of certain chemical manufacturing processes (Carey & Hart 1986). HCBD proved moderately reactive. At a hematin concentration of 1.0 mg/mL, its concentration decreased by about one half in 24 hours and by about 90% in two weeks. No products could be detected.



Octachlorostyrene (OCS, XIII) is also a by-product of the chemical industry, occurring as a contaminant in industrial areas (Kaminsky & Hites 1984; Kuehl et al. 1980; ten Noever de Brauw & Koeman 1972/73; Carey & Hart 1986). It is believed that it was produced by the chlorination of the binder in graphite electrodes used in the electrolytic production of chlorine (Kaminsky & Hites 1984). It appears to undergo very slow dechlorination with hematin at a concentration of 1.0 mg/mL; after about two weeks, a small quantity of product could be detected with a mass spectrum showing a seven-chlorine cluster at $m/z = 342$ ($C_8HCl_7^+$) as well as clusters at 307, 272 and 237, apparently resulting from the loss of three successive chlorine atoms. This is very similar to spectra that have been attributed to heptachlorostyrene isomers (Kaminsky & Hites 1984; Kuehl et al. 1980; ten Noever de Brauw

& Koeman 1972/73; Carey & Hart 1986). this does not appear to have been a contaminant in the octachlorostyrene sample because no trace could be found in the starting material.



A number of aromatic compounds were tested. These included 2-, 3-, and 4-chlorobenzoic acid; 2,5-, 3,4-, and 3,5-dichlorobenzoic acid; three commercial polychlorinated biphenyl (PCB) mixtures, Aroclor 1221, Aroclor 1242, and Aroclor 1254; and two individual chlorobiphenyls, 4,4'-dichlorobiphenyl and decachlorobiphenyl. None of these showed any evidence of reacting over periods of up to two weeks.

Structure-activity relationships for the reactivity of organochlorine compounds with reduced iron porphyrins are still only imperfectly understood. Klecka & Gonsior (1984) suggested, on the basis of their studies on the reaction of reduced porphyrins with a number of chlorinated hydrocarbons, that the reaction is limited to aliphatic molecules with three or more chlorine atoms on the same carbon atom. It appears, however, that the number of chlorine atoms may be less important in determining the ability to react than the degree of saturation, since DDD was found to react, although more slowly than DDT, while DDE did not. The highly unsaturated compounds DCBD and OCS reacted much more slowly than DDT and methoxychlor. Aromatic compounds seem to be totally unreactive. Among alicyclic compounds other principles appear to apply and not enough information is available to warrant any generalizations, except perhaps that epoxides tend to be less reactive than their parent chlorohydrocarbons.

A comparison of reactivity with iron porphyrins with behaviour in the environment is more interesting. Except with mirex, which is quite reactive but extremely persistent in the environment, the relative reactivities within any group of compounds (DDT and analogues, BHC isomers, and bridged chlorinated hydrocarbons) agree fairly well with what might be expected from their relative persistences. It seems that reactivity with reduced hematin may be useful as a predictor of a compound's likely stability under anoxic conditions in nature.

ACKNOWLEDGEMENTS

I am very grateful to Mr. J.H. Hart for obtaining the mass spectra discussed in this paper.

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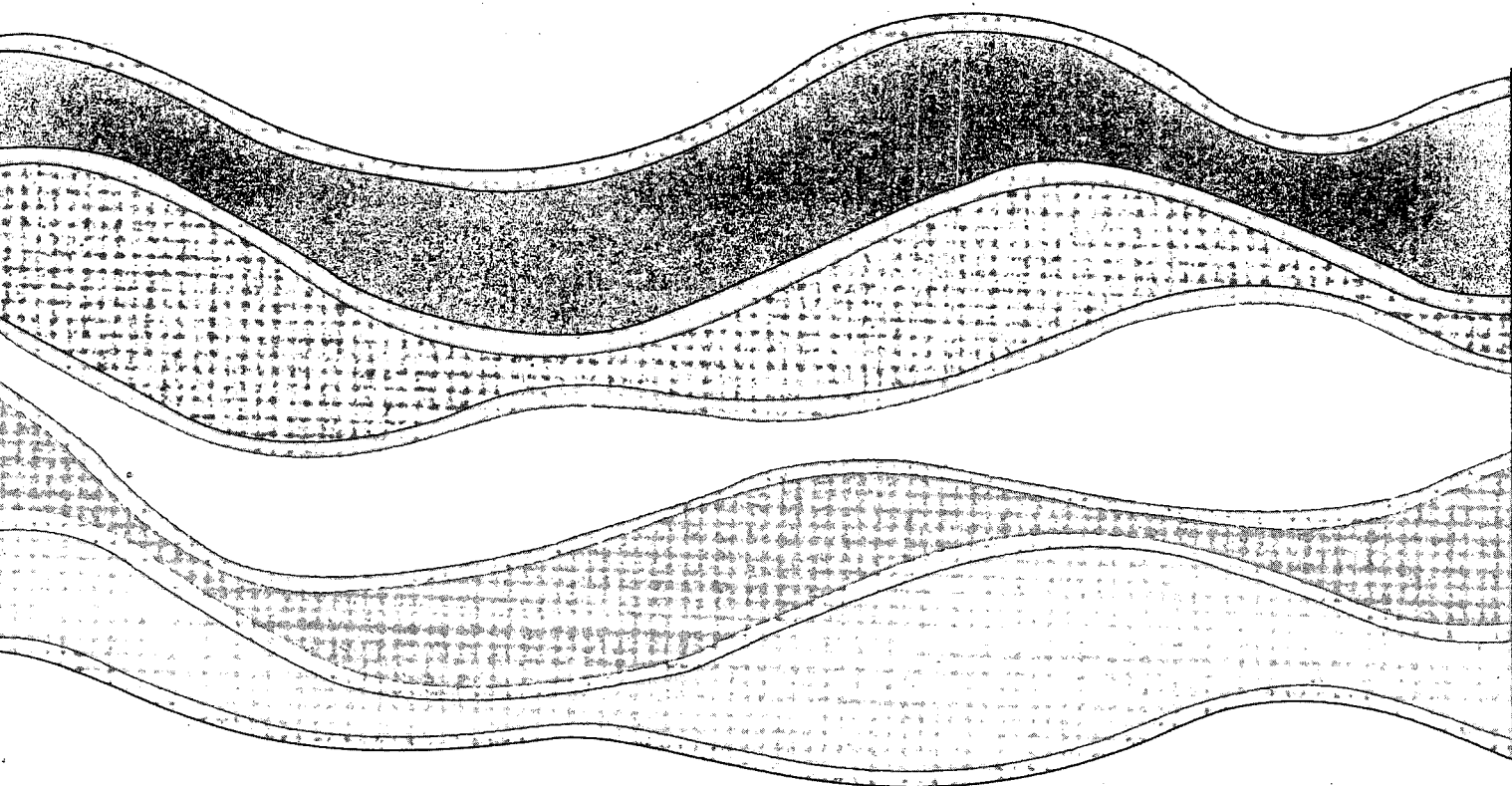
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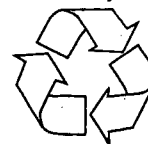
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