Free Radical Mechanism for Solvent Toxicity

ZOLTON T. WIRTSCHAFTER, MD
AND
MARSHALL W. CRONYN, PhD
PORTLAND, ORE

Free Radicals in Solvent Toxicity

A previous study (Wirtschafter and Cronyn, 1963) reported the relative toxicity of molar equivalent doses of several halogenated and nonhalogenated hydrocarbons. Previous investigators have studied the relative toxicity within homologous series, but there have been no quantitative comparisons reported for different types of solvents. von Oettingen et al (1949) made a thorough study of the relative activity of the halogenated methanes; Plaa et al (1958) extended their comparisons from halogenated methanes to ethanes and halogenated ethylenes; and Gerarde (1956, 1959) investigated the relation between structure and toxicity in a large number of alkylbenzenes. The results of our studies and those previously reported are consistent with the hypothesis that the primary step in the biological attack on all such compounds is a free radical process. The toxicity of these solvents is best explained by their reaction with the free radicals which are normal intermediates in many of the most important biological processes.

A free radical bond-breaking process is one in which a pair of electrons bonding two atoms is broken in such a way as to divide the pair evenly between the two atoms, rather than leaving both electrons with one atom or the other, ie:

$$A:B \longrightarrow A\cdot + B\cdot$$

or $RO\cdot + A:B \longrightarrow R\text{-}O:A + B\cdot$

or for a double bond $A::B \longrightarrow \cdot A:B\cdot$

$RO\cdot + A::B \longrightarrow RO:A:B\cdot$

Thus, the radical initiated polymerization of ethylene proceeds as follows:

$$RO\cdot + C::C \longrightarrow RO:C:C\cdot + C::C \longrightarrow RO:C:C:C:C\cdot \longrightarrow \text{etc}$$

and when a molecule such as CCl_4, which is susceptible to radical attack, is added, it may be attacked by the radical end of the growing chain (Walling, 1957);

$$Cl:\dot{C}:Cl + R^1\cdot \longrightarrow R^1:Cl + Cl:\dot{C}\cdot$$

trichloromethyl radical

In these examples, RO· may be a ready source of free radicals such as from the breaking of the very labile peroxide bond:

$$R\text{-}O:O\text{-}R \xrightarrow[\text{or light}]{\text{heat}} 2R\text{-}O\cdot$$

Early studies by Michaelis (1938, 1951) on biological oxidation-reduction reactions very strongly implicated free radicals as intermediates; more recently, the action of ionizing, ultraviolet, and visible radiations on biological systems has also produced evidence of the free radical nature of the reactions produced by these energy sources (Weiss, 1946). Finally, Commoner et al (1954) have succeeded in measuring by electron spin resonance technique the actual concentrations of free radicals in a variety of biological tissues, and Ternberg and Commoner (1963) have applied these techniques to the differential diagnosis of jaundice.

Submitted for publication Oct 11, 1963.

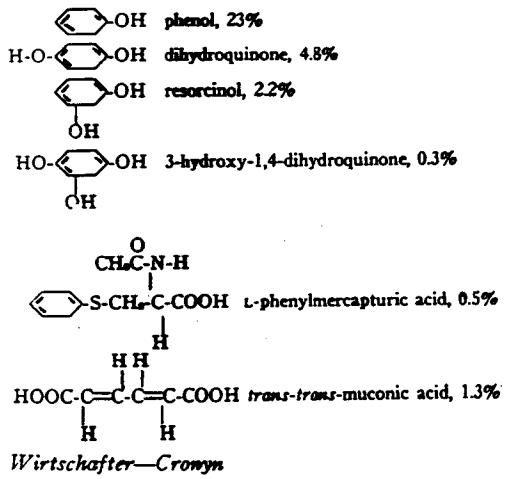
Research Laboratory of the Veterans Administration Hospital, the University of Oregon Medical School, and the Department of Chemistry, Reed College.

It is apparent from a review of the literature on solvent toxicity that no satisfactory theory of action has been suggested which would cover equally well both halogenated and nonhalogenated toxicity. However, it is quite apparent from all of the results so far reported that the products obtained in the action of a biological system on hydrocarbons or their halogenated derivatives are the same as the products obtained when the same compounds are subjected directly to free radical attack in an aqueous medium.

Biochemical Breakdown of Solvent Molecules

Benzene, nitrobenzene, and the three dichlorobenzenes yield phenolic products whether metabolized in the rabbit (Parke and Williams, 1953[21,22]; Azouz et al, 1955; Parke, 1956), subjected to radiation in aqueous solution (Stein and Weiss, 1949), or treated with a metal ion plus peroxide source of free radicals (Konecny, 1954). Stein and Weiss (1949), Parke and Williams (1953[21,22]), Azouz et al (1955), Parke (1956), Baernstein (1945), and Bernhard (1941) pointed out the similarity between their radiation products and the phenols obtained when the same compounds were subjected to biological metabolism.

Within 48 hours, 45% of the total benzene had been expired, 43% as unchanged benzene and 1½% as CO_2. At the end of the 48-hour period, 35% of the benzene appeared in the form of urinary metabolites of the following types:



The phenolic products indicate quite clearly that the benzene ring is being attacked by H-O· or H-O-O· radicals in the same manner that has been observed when benzene or benzene derivatives are treated with Fenton's reagent (Fe^{++} and H_2O_2):

$$C_6H_6+H_2O_2+Fe^{++}\longrightarrow C_6H_5OH+H_2O$$

or Cu^{+} ion and H_2O_2 (Konecny, 1954).

Similarly, when benzene is irradiated in aqueous solution in the presence of oxygen, phenols are produced by the attack of radicals generated by the action of the radiation on the water (Bacq and Alexander, 1955).

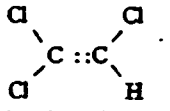
$$H_2O+O^{x}\longrightarrow\cdot OH+H\text{-}O\text{-}O\cdot$$
$$ArH+\cdot OH\longrightarrow Ar\cdot+H_2O$$
or $$ArH+\cdot O\text{-}O\text{-}H\longrightarrow Ar\cdot+H_2O_2$$
$$Ar\cdot+\cdot OH\longrightarrow ArOH$$

(Stein and Weiss, 1949)

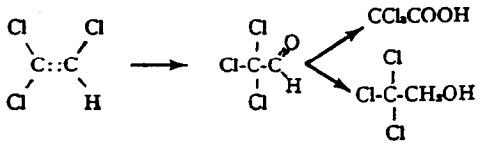
The finding by Gerarde (1956) that alkylbenzenes are much less myelotoxic than benzene provides another indication that the metabolism of the hydrocarbons is initiated by a free radical attack. The radical attack on alkylated benzene generally proceeds at the methylene next to the aromatic ring rather than on the ring itself. The resulting radicals have a much greater stability than those which would result from the removal of a ring hydrogen.

$$ArCH_3+\cdot OH\longrightarrow Ar\text{-}CH_2+H_2O$$

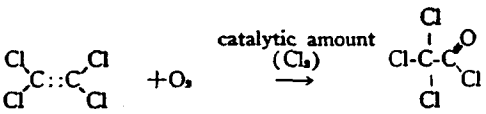
Harper and Calcutt (1961) have found that the hydroxylation of polynuclear hydrocarbons is promoted by a microsomal fraction of liver homogenate and is inhibited by riboflavin. In Gerarde's work on the alkylbenzenes (1956), the primary products were found to be hydroxy derivatives with the major substitution occurring on the C-H adjacent to the aromatic ring. In barbiturates, also, the reaction products indicate side chain hydroxylation followed by oxidation to carboxyl acids (Brodie, 1956; Maynert and Losin, 1955). Butler (1949) has found that the metabolism of trichloroethylene,



in dogs, gives chloral, which is further metabolized to trichloroethanol and trichloroacetic acid.



The conversion of trichloroethylene to chloral finds a parallel in the free radical mediated radiation conversion of tetrachloroethylene into trichloroacetyl in the presence of oxygen (Walling, 1957).



The fate of $C^{14}Cl_4$ absorbed by inhalation in monkeys has been studied by McCollister et al (1951). They have found that 40% of the $C^{14}Cl_4$ is exhaled in 1,800 hours as $C^{14}Cl_4$ and 11% as $C^{14}O_2$. The remaining C^{14} was found as carbonate and nonvolatile bound C^{14} in urine and feces. In the case of CCl_4, unlike benzene and trichloroethylene, there are two processes by which the molecule might be degraded to CO_2, the free radical path, such as observed by Minder (1948), who measured the HCl generated when CCl_4 was exposed to x-rays in aqueous solution and the nucleophilic decomposition which has been studied by W. F. von Oettingen et al (1949). However, the relative toxicities of the various halogenated methanes do not seem to relate to the large difference in nucleophilic reactivity between chloroform and carbon tetrachloride.

Recently, Butler (1961) has determined that CCl_4 is converted into $CHCl_3$ both in vivo and in vitro by various tissues and tissue constituents and has proposed that in certain halogenated hydrocarbons such as CCl_4 and $CHCl_3$, toxicity is the result of a free radical cleavage and the subsequent chemical fate of the radical products. Butler limits his suggestion to halogenated hydrocarbons and even more strictly to those in which the C-Cl dipole is reduced to lower the activation energy for a homolytic rather than heterolytic cleavage of the carbon-halogen bond.

We believe that the notion of a homolytic process as Butler has suggested is an excellent one, since it not only explains his observed reduction, but it provides a clue to the behavior of other solvents in biological systems. Furthermore, Commoner et al (1954) demonstrated the presence of free radicals in *biological* material at a level of about 10^{-8} mol/gm. Because Butler (1961) correlated the potential free radical activity of CCl_4 and $CHCl_3$ with their C-Cl dipole moment and the activation energy for C-Cl bond splitting as a unimolecular process, he did not extend his proposal to simple hydrocarbons and less highly substitute halogenated derivatives.

However, we would like to suggest that it may be more profitable to analyze the relative propensity for free radical formation using another criterion, namely the relative rate of reaction of various hydrocarbons and their halogenated derivatives directly with free radicals already present in the medium. In other words, to consider this as at least a bimolecular (or higher) process at the bond-breaking step; ie,

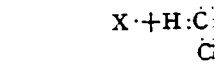
$$X\cdot + A:B \longrightarrow X:A + B\cdot$$
rather than $A:B \longrightarrow A\cdot + B\cdot$

The best data for this type of comparison are to be found in the transfer constants which measure the relative rate of attack of a growing free radical catalyzed polymer chain on solvent molecules compared to their rate of attack on more of the monomer units (Walling, 1957).

The advantage in using this type of system as a measure of relative energy for free radical formation is apparent if we examine the behavior of $CHCl_3$. If we assume that under a unimolecular process the weakest bond will be broken, then the C-Cl bond should cleave since the bond energies in $CHCl_3$ are C-Cl, 72 k-cal/mol and C-H, approximately 90 k-cal/mol (Cottrell, 1958).

However, when $CHCl_3$ actually reacts by a free radical process, such as with polymerizing 1-octene, the ratio of C-H to C-Cl bond cleavage is about 50 or 60 to 1 (Walling, 1957). Thus, in spite of the much greater bond strength for the C-H bond, it is preferentially cleaved by a free radical. The reason for this is that in a reaction with

another radical, the coupled with a bo the activation ener point in this proce the isolated bond- energy of partial b favorable interact tronic configuratic and the new radic



These character would account for CH_2Cl_2 found by experiments and vivo. It would also of CCl_4 which is to CO_2 (McCol though the first st pears to be a red lowed through t CH_2Cl_2, CH_3Cl s of CO_2.

That the *relativ* different bonds, s vary with the sou demonstrated in stants of CCl_4 and tion of several all

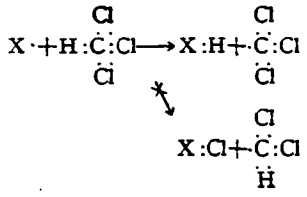
An examinatio for CCl_4, benzen clearly that CCl_4 radical reactor a ference between hydrocarbons.

The implicatio proposal and the

TABLE 1.—*Transfer*

Radical Source
Styrene (60 C)
Vinyl acetate (60 C)
Ethylene (70 C)

another radical, the bond-breaking process is coupled with a bond-forming process, and the activation energy required for the midpoint in this process is decreased relative to the isolated bond-breaking process both by energy of partial bond formation and by any favorable interactions between other electronic configurations of the radical source and the new radical being generated.



These characteristics of radical reactions would account for the very small amount of CH_2Cl_2 found by Butler (1961) for in vitro experiments and account for none at all in vivo. It would also explain the oxidative fate of CCl_4 which is converted, in part at least, to CO_2 (McCollister et al, 1951) even though the first step observed by Butler appears to be a reductive process, and if followed through the series, CCl_4, $CHCl_3$, CH_2Cl_2, CH_3Cl should lead to CH_4 instead of CO_2.

That the *relative* ease of bond breaking for different bonds, such as C-H and C-Cl, will vary with the source of the radical is clearly demonstrated in the relative transfer constants of CCl_4 and $CHCl_3$ in the polymerization of several alkenes (Walling, 1957).

An examination of the transfer constants for CCl_4, benzene, and cyclohexane shows clearly that CCl_4 is by far the most effective radical reactor and that there is little difference between benzene and the saturated hydrocarbons.

The implications of these results for our proposal and the comparison of the biological activity of various solvents is apparent. Since the relative rate of attack within a homologous series of hydrocarbons or halogenated hydrocarbons for a radical process will depend on the nature of the attacking radical, we may not expect to find a very clear parallel between any simple chemically determined relative order of activity and a biological one. Not only is there no single chemically determinable reference order of activity, but in the biological system, the relative order will vary with the many possible radical sources within the living system. The combination of differences in activity as a function of the radical generating entity and the differences in tissue distribution (von Oettingen et al, 1949; Gerarde, 1956; Minder et al, 1948), due in part to differences in distribution coefficients and vapor pressure (von Oettingen et al, 1949; Gerarde, 1956), is no doubt responsible for the observation that there is no correlation between the dose causing liver damage and that causing death. Thus, a comparison of the hepatotoxic effects at ED_{50} (the dose producing the desired effect in 50% of the cases) shows that CCl_4 is more toxic than $CHCl_3$. The ED_{50} values in millimols per kilogram were determined by Plaa et al

TABLE 1.—*Transfer Constants for CCl_4 and $CHCl_3$*

Radical Source	Transfer Constants	
	CCl_4	$CHCl_3$
Styrene (60 C)	90×10^{-4}	0.5×10^{-4}
Vinyl acetate (60 C)	1.0	0.016
Ethylene (70 C)	0.7	0.8

TABLE 2.—*Transfer Constants*

Solvent	Styrene With Typical Solvent *	
	60 C	100 C
Cyclohexane	0.0000024	0.000016
Benzene	0.0000018	0.000018
Methylene chloride	0.000015	
Chloroform	0.000050	
Ethylene dichloride		0.00066
Tetrachloroethane		0.0018
Carbon tetrachloride	0.0090	0.0180
n-Butyl mercaptan	22	

		Ethylene † 70 C
Methyl chloride	CH_3Cl	0.0004
Methylene chloride	CH_2Cl_2	0.07
Chloroform	$CHCl_3$	0.8
Carbon tetrachloride	CCl_4	0.7
Ethyl chloride	CH_3CH_2Cl	0.012
Ethylidene dichloride	$CH_3CH\,Cl_2$	0.15
Methyl chloroform	CH_3CCl_3	0.05

* Walling, reference 28, p 152.
† Walling, reference 28, p 257.

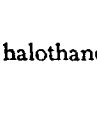
(1958) to be 1.4 for $CHCl_3$ and 0.45 for CCl_4, whereas for the lethal dose the reverse is true: LD_{50}, 5.9 for $CHCl_3$ versus 200 mM/kg for CCl_4. This is also the order of toxicity observed by von Oettingen et al (1949) for the lethal effect when both compounds were administered at the same concentration.

Where the structures being compared constitute a homologous series or a group of compounds of very similar structure, but without a wide range of solubility characteristics, the order of reactivity correlates more often than not with the order to be expected for a free radical process in which there is a radical attack upon the hydrocarbon or its derivative. For example, Plaa et al (1958), using a sensitive test for hepatotoxicity, found the following ED_{50} values (mM/kg): CCl_4 (carbon tetrachloride)-0.45; $CHCl_3$ (chloroform)-1.40; $CH_2ClCHCl_2$ (1,1,2-trichloroethane)-2.1; $CHCl_2CHCl_2$ (*sym*-tetrachloroethane)-7.2; $CHCl{=}CCl_2$ (trichloroethylene)-11; $CCl_2{=}CCl_2$ (tetrachloroethylene)-27; CH_3CCl_3 (1,1,1-trichloroethane)-84. If the unsaturated compounds are considered separately, since they would be expected to react by radical additions, the others in this series correspond quite well to the expected reactivity toward a free radical source. Between the two unsaturated compounds, the order is also what would be expected on this basis (Walling, 1957).

Gerarde (1956) rated alkylbenzenes according to their lethal effect for rats. There is a similarity between the transfer constant pattern and the lethal ratio (Table 3). In the unbranched side chain series, the effect of solubility is apparent since the toxicity rises to a maximum and then drops off to 0/10 for *n*-decylbenzene.

TABLE 3.—*Transfer Constants and Lethal Ratios for Alkylbenzenes*

	Transfer Constant Styrene (60 C)	Lethal Ratio
Benzene	0.24×10^{-4}	0/10
Toluene	1.25×10^{-4}	3/10
Ethylbenzene	6.70×10^{-4}	7/10
Isopropylbenzene	8.20×10^{-4}	6/10

It is not surprising that the new anesthetic, halothane,  , has caused liver damage since it has one hydrogen which should be readily removed under free radical attack. It should also be expected that compounds other than hydrocarbons and their halogenated derivatives may produce similar toxic effects if they are highly susceptible to attack by free radicals and are not metabolized in some other way. For example, mercaptans have one of the highest transfer constants (Walling, 1957), being about 10^4 times as effective as carbon tetrachloride in styrene polymerization, so that in addition to any possible disruption of physiological processes via $-SH$ exchange, complexing with metal ions, etc, radical formation may give rise to further toxic effects. It should also be expected that compounds which are presumed to be useful for their antiradiation effects may be expected to show disruption of normal processes as a consequence of their particular reactions with radicals.

Aminoethylthiourea was studied by Doherty and Burnett (1955) and found to have considerable protective effect, showing a survival rate of 70% at day 28 in mice subjected to a lethal dose of x-irradiation. Cheymol et al (1959) have tested aminoethylthiourea as to its effect on dehydrogenase activity of the liver of the mouse subjected to x-irradiation. They determined that γ-radiations (900 r) not only do not inhibit succinodehydrogenase activity of the liver of the mouse, but on the contrary, they appear to increase it. They further demonstrated that aminoethylthiourea exercises the same effect on the enzymatic system as do the γ-radiations and that it does not compensate for the ill-effects caused by the radiation.

Summary

We believe that our own results and all of the evidence previously reported on the

relative toxicities and metabolic products for the entire range of hydrocarbon and halogenated hydrocarbon solvents are entirely consistent with the hypothesis that the mechanism by which these molecules are attacked in the biological system involves an initial free radical process.

It is clear that the thermodynamic value for a homolytic cleavage is not as useful an index of reactivity as the transfer constant based on observations of the relative frequency with which a particular molecule is attacked by a growing free radical propagated polymer. There are several other factors which influence the toxic effectiveness of a particular compound and consequently lower the probability that direct correlation can be made. These factors are: (1) For any series of solvents, different relative rates of reaction are to be expected for different radical sources; (2) water/lipid distribution coefficients are significant; (3) vapor pressure is important.

Zolton T. Wirtschafter, MD, Research Laboratory, VA Hospital, Sam Jackson Park Rd, Portland, Ore 97207.

REFERENCES

1. Azouz, W. M.; Parke, D. V.; and Williams, R. T.: Biochem J 59:410, 1955.
2. Bacq, Z. M., and Alexander, P.: Fundamentals of Radiobiology, New York: Academic Press, Inc., 1955.
3. Baernstein, H. D.: J Biol Chem 161:685, 1945.
4. Bernhard, K., and Gressly, E.: Helv Chim Acta 24:83, 1941.
5. Brodie, B. B.: J Pharm Pharmacol 8:1, 1956.
6. Butler, T. C.: J Pharmacol Exp Ther 97:84, 1949.
7. Butler, T. C.: J Pharmacol Exp Ther 134:311, 1961.
8. Cheymol, J., et al: C R Soc Biol (Paris) 153:1965, 1959.
9. Commoner, B.; Townsend, J.; and Pake, G. E.: Nature (London) 174:689, 1954.
10. Cottrell, T. L.: Strength of Chemical Bonds, London: Butterworth Scientific Publications, Inc., 1958.
11. Doherty, D. G., and Burnett, W. T., Jr.: Proc Soc Exp Biol Med 89:312, 1955.
12. Gerarde, H. W.: AMA Arch Industr Health 13:468, 1956.
13. Gerarde, H. W.: AMA Arch Industr Health 19:403, 1959.
14. Harper, K. H., and Calcutt, G.: Nature (London) 192:165, 1961.
15. Konecny, J. O.: J Amer Chem Soc 76:4993, 1954.
16. Maynert, E. W., and Losin, L.: J Pharmacol Exp Ther 115:275, 1955.
17. McCollister, D. D., et al: J Pharmacol Exp Ther 102:112, 1951.
18. Michaelis, L., and Schubert, M. P.: Chem Rev 22:437, 1938.
19. Michaelis, L., in The Enzymes, J. B. Sumner and K. Myrback, ed., New York: Academic Press, Inc., 1951, vol 2, pt 1. chap 44.
20. Minder, W.; Knuchel, H.; and Gurtner, P.: Experientia 4:219, 1948.
21. Parke, D. V., and Williams, R. T.: Biochem J 54:231, 1953.
22. Parke, D. V., and Williams, R. T.: Biochem J 55:337, 1953.
23. Parke, D. V.: Biochem J 62:339, 1956.
24. Plaa, G. L.; Evans, E. A.; and Hine, C. H.: J Pharmacol Exp Ther 123:224, 1958.
25. Stein, G., and Weiss, J.: J Chem Soc, pt 4, suppl 1-2:3245, 1949.
26. Ternberg, J. L., and Commoner, B.: JAMA 183:339, 1963.
27. von Oettingen, W. F., et al: National Institutes of Health Bulletin No. 191, United States Public Health Service, 1949, p 1.
28. Walling, C.: Free Radicals in Solution, New York: John Wiley & Sons, 1957.
29. Weiss, J.: Nature (London) 157:584, 1946.
30. Wirtschafter, Z. T., and Cronyn, M. W.: Relative Hepatotoxicity, this issue, pp 180-185.