The microsomal metabolism of the organometallic derivatives of the Group-IV elements, germanium, tin and lead

Russell A. PROUGH,* Mary Anne STALMACH,* Philip WIEBKIN* and James W. BRIDGES†

*Department of Biochemistry, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX75235, U.S.A., and †Robens' Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

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The NADPH- and oxygen-dependent microsomal metabolism of the di-, tri- and tetra-ethyl-substituted derivatives of germanium, tin and lead was shown to give rise to ethylene as a major product and ethane as a minor product. These reactions were shown to be catalysed by the liver microsomal cytochrome *P*-450-dependent mono-oxygenase. Since formation of ethane and ethylene was differentially inhibited by anaerobiosis, the results suggest that at least a large portion of the ethane produced may be derived by a reductive mechanism. Triethyltin bromide in both the absence and presence of NADPH was shown to convert cytochrome *P*-450 into cytochrome *P*-420 and to affect the function of the mono-oxygenase *in vitro*. Tetraethyltin caused the NADPH- and time-dependent formation of cytochrome *P*-420, suggesting that tetraethyltin is converted into triethyltin salts in significant concentrations. The order of potency in formation of cytochrome *P*-420 was closely paralleled by the ability of the tin derivatives to induce microsomal lipid peroxidation *in vitro*.

Considerable interest in Group-IV organometallic derivatives has arisen owing to their limited, but significant, use in agriculture and industry and their unique toxicity in mammals (Barnes & Stoner, 1959; Gitlitz, 1976). Their beneficial application in society has been widespread in the plastics industry, in pesticide control in agriculture, in prevention of 'knock' in the internal combustion engine, and as antifoulants for marine vessels (Barnes & Magos, 1968; Barnes & Stoner, 1959; Roller, 1970). Since the tragic human exposure to triethyltin salts in France (Barnes & Stoner, 1959), the toxic and biochemical effects of many of these derivatives have been explored. Di- and tri-ethyltin salts have been demonstrated to have pronounced effects on intermediary metabolism in brain and liver (Aldridge, 1976; Aldridge & Cremer, 1955; Cremer, 1957, 1958). These effects have been suggested to be due to inhibition of mitochondrial function at the level of α -oxo acid oxidation (diethyltins) or electron transfer (triethyltins) within the mitochondrial electron-transport chain. Owing to the particular sensitivity of oxygen metabolism in brain to triethyltin compounds, it is assumed that the brain oedema caused by these compounds is most probably related to brain mitochondrial function and ion transport.

Cremer (1957, 1958) documented that liver microsomal protein most probably was involved in the conversion of tetraethyltin into its triethyltin salt, leading to the brain toxicity, and further suggested that the triethyl derivative is not metabolized to the diethyltin salt to any significant extent. Dealkylation of organotin was also shown by Bridges *et al.* (1967), who reported the conversion of diethyltin salts into monoethyltin salts *in vivo* and *in vitro*. It has also been shown that the tetra-alkyl-lead derivatives can also be converted into their tri-alkyl-lead salts (Bolanowska & Wisniewska-Knypl, 1971; Stevens *et al.*, 1961). Casida and co-workers (Casida *et al.*, 1971; Fish *et al.*, 1976, 1977), have studied the *C*-hydroxylation of a number of butyl- and cyclohexyl-substituted tin derivatives by liver microsomal fractions and have demonstrated the involvement of the cytochrome *P*-450-dependent mono-oxygenase in these reactions. In most cases, stable alcohol derivatives of the organometallic compound were formed, but with tributyltin acetate, butane was observed as one product.

The present paper documents the volatile products formed by dealkylation of the ethyl-substituted organometallic derivatives of germanium, tin and lead with liver microsomal fractions, oxygen and

NADPH. In addition, the effect of these compounds on the function of cytochrome *P*-450 *in vitro* will be described.

Materials and methods

All of the organometallic compounds except for tetraethyl-lead were purchased from Alpha Products, Thiokol Ventron Division, Danvers, MA, U.S.A., and used without further purification. Tetraethyl-lead was supplied as a 1.0M solution in ethanol by Associated Octel Ltd., Runcorn, Cheshire, U.K. NADP⁺, NADPH, NADH, sodium isocitrate, isocitrate dehydrogenase, catalase, glucose oxidase and Trizma base were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other laboratory chemicals were purchased from commercial sources in reagent-grade purity.

Male Sprague-Dawley rats (200–300g) were purchased from Charles River Co., Wilmington, MA, U.S.A., and maintained *ad libitum* on laboratory chow and water. The rats were pre-treated with either phenobarbital in saline (80mg/kg for 4 days) or 5,6-benzoflavone in corn oil (50mg/kg for 4 days) and starved for 18h before being killed. Liver microsomal fractions were prepared as described by Remmer *et al.* (1967) and the protein concentration was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

A standard 10ml reaction mixture containing 0.8 unit of isocitrate dehydrogenase/ml, 5mM-sodium isocitrate, 1.5mM-MgCl₂, 1mg of microsomal protein/ml and 0.1M-Tris/HCl buffer, pH 7.6, was used for all metabolism studies. The reaction was initiated by addition of microsomal protein followed by substrate after a 5 min preincubation at 37°C. Benzphetamine metabolism to formaldehyde was measured by the method of Nash (1953), and ethoxycoumarin and ethoxyresorufin dealkylation were measured fluorimetrically as described by Prough *et al.* (1978). NAD(P)H-cytochrome *c* reductase activity was measured by the method of Masters *et al.* (1967). Lipid peroxidation was measured as malonaldehyde formation from microsomal lipids (Buege & Aust, 1978).

The evolution of ethane and ethylene was measured in closed 25ml Erlenmeyer flasks sealed with rubber serum stoppers; the gas-phase volume was 19ml. Samples of the gas phase were removed with a 1ml gas-tight syringe and injected on to a Porapak-Q column at 80°C in a Hewlett-Packard model 5750B gas-liquid chromatograph equipped with a flame ionization detector. The detector response was calibrated by using standard dilutions of ethane and ethylene. The organometallic com-

pounds were dissolved in reagent-grade acetone and added in small volume to give a final concentration of 2mM. The rate of hydrocarbon formation was obtained by plotting 0, 5, 10 and 15 min time points and measuring the slope of the plot. Normally these plots were linear for over 15 min. When anaerobiosis was desired, catalase (2 units/ml) and glucose oxidase (7 units/ml) were added to the reaction mixture and the solution was bubbled with argon. The flask was sealed with the serum stopper, glucose (10mM) was added by syringe and the preincubation period begun. After 5 min, it could be demonstrated that the solution was totally anaerobic. Experiments with sodium dithionite were performed in the presence and absence of oxygen in 0.1M-Tris/HCl buffer, pH 7.6, and the reaction was initiated by addition of reducing agent. Content of cytochromes *P*-450 and *P*-420 was measured with an Aminco DW2 spectrophotometer by the method of Omura & Sato (1964).

Results

Volatile products of ethyl-substituted organometallics

As suggested by Bridges *et al.* (1967), it was decided to determine whether any alkanes or alkenes were formed during the microsomal metabolism of triethyltin salts. Using a Porapak Q g.l.c. column support, one can adequately separate and quantify the lower alkanes and alkenes with a gas-liquid chromatograph equipped with a flame ionization detector (Fig. 1). The evolution of alkanes and alkenes from microsomal reaction mixtures containing alkyltins was measured in closed vessels sealed with serum stoppers by analysing samples of the gas phase by g.l.c. (Prough *et al.*, 1969). The two gaseous metabolites formed by the microsomal metabolism of triethyltin bromide can be resolved by this method and have retention times identical with those of ethylene and ethane standards. When tributyltin bromide was utilized as a substrate, no ethane or ethylene was formed as products, but butane and butene were formed in appreciable quantities (results not shown).

Characterization of ethane and ethylene formation

In order to characterize the enzyme system involved in the dealkylation of triethyltin bromide, the conditions of the microsomal reaction mixture were varied. The reactions were performed at 37°C and the assay was optimized for time, protein, pH and substrate concentration for triethyltin bromide ($K_m \approx 0.3$ mM). The formation of ethane and ethylene was linear with time to 15 min and with protein to 4mg/ml; the optimal pH appeared to be 7.6. The

Fig. 1. Separation of the lower alkanes by g.l.c. A Porapak-Q column support was used at 80°C and the separation monitored with a flame ionization detector. (a) Separation of methane, ethylene, ethane and propane. (b) Separation of the gaseous metabolites of triethyltin bromide (2mM) formed at 10min by liver microsomal protein from phenobarbital-treated rats (1mg/ml), NADPH and oxygen. The peaks apparent at 0.5–0.7min retention time were present before initiation of the reaction.

metabolic rate of ethylene formation at 37°C was 0.20 ± 0.04 (s.d.) nmol/min per mg of microsomal protein from phenobarbital-treated rats ($n = 5$). A negligible amount of ethylene (1–5% of control) was formed if either liver microsomal fractions or NADP⁺ (or NADPH) were omitted. NADH only supported the reaction at approx. 15% of the rate with NADPH. When the reaction mixture was made anaerobic before initiation of the reaction, the rate was decreased to 15% of control. The formation of ethane was similarly effected by the changes described above, and these results suggest that a NADPH- and oxygen-dependent oxygenase is involved.

Since cytochrome *P*-450 is thought to be the major oxidative enzyme in microsomal fractions involved in metabolism of xenobiotic compounds, we tested the effects of several known inhibitors of cytochrome *P*-450 or NADPH–cytochrome *P*-450

Table 1. Influence of various inhibitors on the formation of ethylene and ethane from triethyltin bromide

The reaction mixture contained liver microsomal protein (1mg/ml) from phenobarbital-treated rats, triethyltin bromide (2mM), and a NADPH-regenerating system. The inhibitors were added before the addition of the microsomal protein and triethyltin bromide. Duplicate samples of the gaseous products were collected in gas-tight syringes and analysed by g.l.c. (see the Materials and methods section). The rates of ethylene and ethane formation in reaction mixtures without inhibitors were 0.27 and 0.034 nmol of hydrocarbon produced/min per mg of microsomal protein respectively.

Compound	Inhibition (%)	
	Ethylene	Ethane
SKF 525A (0.2mM)	45	50
Metyrapone (0.2mM)	72	48
Octylamine (2.0mM)	80	70
Anti-(NADPH–cytochrome <i>c</i> reductase) globulin	91	85
Non-immune globulin	1	4
CO/O ₂ (4:1, v/v)	70	65

reductase on hydrocarbon formation from triethyltin bromide (Table 1). SKF-525A, metyrapone, octylamine and a gas atmosphere of CO/O₂ (4:1) all caused marked inhibition of the reaction, and the inhibitory antibody to NADPH–cytochrome *P*-450 reductase inhibited ethylene formation by 91%. Non-immune globulin had no effect on the reaction. Similar results were seen on the formation of ethane (Table 1). These results strongly suggest that the microsomal cytochrome *P*-450-dependent mono-oxygenase is involved in hydrocarbon formation from triethyltin salts. It was also noted that metyrapone and octylamine inhibited ethane and ethylene formation when the reaction was performed under anaerobic conditions (results not shown).

Hydrocarbon formation from other ethyl-substituted organometallic derivatives of the Group-IV elements

Table 2 demonstrates that a number of other ethyl-substituted metals at one common substrate concentration are metabolized to give ethane and ethylene as products. It is notable that tetraethyl-lead and -tin form more ethylene than do the triethyl derivatives, suggesting that there may be a correlation between the rate of metabolism and lipophilicity (and perhaps non-metallic character). However, the germanium compounds did not follow such an order, and ethane production from these compounds was barely detectable.

Table 2. *Metabolism of various organometallic compounds to ethylene and ethane*

The reaction mixture contained liver microsomal protein (1mg/ml) from phenobarbital-pretreated rats, a NADPH-regenerating system, and the organometallic compound (2 mM). Duplicate samples of the hydrocarbons were collected in gas-tight syringes and the products analysed by g.l.c. as described in the Materials and methods section. The rates are expressed as nmol of hydrocarbon formed/min per mg of microsomal protein. The values were averages of three experiments with a standard deviation of less than 10% of the average.

Compound	Metabolic rate	
	Ethylene	Ethane
Tetraethyl-lead	5.20	0.36
Triethylgermanium chloride	1.60	≤0.004
Tetraethyltin	0.86	0.020
Triethyl-lead acetate	0.51	0.030
Triethyltin bromide	0.28	0.036
Tetraethylgermanium	0.13	<0.004
Diethyltin dichloride	<0.01	<0.002

Effect of animal pretreatment on the dealkylation of triethyltin bromide

To characterize the reaction further, animals were pretreated with either phenobarbital or 5,6-benzoflavone, and the ability of microsomal preparations to convert triethyltin salts into ethane and ethylene was studied. As shown in Table 3, animal pretreatment with phenobarbital caused a 7-fold increase in ethylene formation, but only a 1.5-fold increase in ethane production when metabolism occurred in the presence of oxygen. Animal pretreatment with 5,6-benzoflavone caused a suppression of the rates of triethyltin dealkylation. It is specially noteworthy that substantial changes in the product ratio (ethylene/ethane formed) occurred after animal pretreatment with phenobarbital; the ratio changes from 0.8 to 3.6, suggesting that the form of cytochrome P-450 that is induced by phenobarbital administration preferentially forms ethylene rather than ethane. Administration of 5,6-benzoflavone *in vivo* does not greatly alter the rates of product ratio of gaseous metabolites formed.

Fig. 2 shows the effect of anaerobiosis on the formation of ethylene from triethyltin bromide. The time courses for ethane formation were similar, but with lower rates of formation. When the reaction mixture was made anaerobic by first gassing the solution and gas space in the closed vessel with argon and then depleting the solution of residual oxygen by addition of glucose, glucose oxidase and catalase to ensure anaerobiosis, the rates of ethylene formation are decreased to a far greater extent than are those for ethane formation. This result is seen

Table 3. *Factors influencing the ratio of ethylene and ethane formed by the metabolism of triethyltin bromide*

The reaction mixtures were identical with those described in Table 1, except that liver microsomal fractions from rats, which were either untreated (CON), treated with phenobarbital (PB), or treated with 5,6-benzoflavone (BF), were used as indicated and oxygen was either absent or present in the gas phase of the closed container. The gas phase was argon and the solution contained a mixture of glucose oxidase, glucose and catalase to maintain anaerobiosis (see the Materials and methods section). The values shown are averages of three experiments with a standard deviation of less than 10% of the average.

Conditions	Source of microsomal fraction	Metabolic rate (pmol/min per mg)		Ethylene/ethane ratio
		Ethylene	Ethane	
Aerobic	CON	31	38	0.8
Anaerobic	CON	6	16	0.4
Aerobic	PB	201	56	3.6
Anaerobic	PB	27	29	0.9
Aerobic	BF	26	26	1.0
Anaerobic	BF	7	13	0.6

Fig. 2. *Time course of ethylene formation in vitro from triethyltin bromide in the presence and absence of oxygen*

The reactions were performed at 37°C under identical conditions (see the Materials and methods section), but the source of liver microsomal protein (1mg/ml) was varied. The concentration of triethyltin bromide used was 2mM. Animal pretreatment regimen: Δ , none; $\circ$, phenobarbital; $\square$, 5,6-benzoflavone.

regardless of the source of microsomal protein. The effect of anaerobiosis on the metabolic rates and product ratio is shown in Table 3. The differences in the product ratio between microsomal protein obtained from animals pretreated with pheno-

barbital and 5,6-benzoflavone are decreased during anaerobiosis compared with those seen in the presence of oxygen. Thus ethane formation is inhibited by only 45–55% during anaerobiosis, as compared with 87–90% for ethylene formation. The differences in the metabolic rates with microsomal protein obtained from animals undergoing various pretreatment regimens and the differences in product ratio between aerobiosis and anaerobiosis imply that there are major differences in either the cytochromes or reactions which give rise to the two metabolic products, ethylene and ethane.

In the light of the possibility that the anaerobic reaction could be due to a reductive process, the ability of added chemical reductants to cause hydrocarbon formation was studied. Both ethylene and ethane could be formed in the presence of sodium dithionite in buffer, and the product ratio (ethylene/ethane) was approx. 30 in the presence or absence of oxygen. These results suggest that chemical reduction of ethyl-substituted organometallics can lead to ethane and ethylene production.

Fig. 3. Effect of triethyltin bromide on cytochrome *P*-450 *in vitro*

Liver microsomal fraction (1 mg/ml) in 0.1 M-Tris/HCl buffer, pH 7.6, was incubated for 10 min in the absence or presence of 1.0 mM-triethyltin bromide at 37°C. The baseline of equal light absorbance was obtained (---) and sodium dithionite was added to both cuvettes. CO was bubbled through the sample cuvette, in the absence (---) or presence of triethyltin bromide (—).

Effect of ethyltin derivatives on microsomal cytochrome *P*-450 and associated enzyme activities

When liver microsomal fractions from phenobarbital-treated rats were incubated at 37°C for 10 min in the presence of 1 mM-triethyltin bromide, the cytochrome *P*-450 was converted into cytochrome *P*-420 (Fig. 3). The time-dependence of the conversion of cytochrome *P*-450 into cytochrome *P*-420 by the tetra- and tri-ethyltin derivatives is shown in Fig. 4. The conversion at 1 mM-triethyltin bromide was complete within 5 min (55% of control cytochrome content), and there was little effect on addition of NADPH (0.25 mM). However, tetraethyltin did not convert cytochrome *P*-450 into cytochrome *P*-420 unless NADPH was added to the reaction mixture. This result is consistent with Cremer's (1957, 1958) observation that liver microsomal fractions convert tetraethyltin into triethyltin salts. In addition, the triethyltin salts formed would appear to be responsible for the formation of cytochrome *P*-420.

The concentration-dependence of this conversion of the cytochrome into cytochrome *P*-420 is shown in Fig. 5. Liver microsomal fractions from 5,6-benzoflavone-treated animals were more sensitive to triethyltin bromide concentration (I_{50} , the concentration of organometallic needed to convert 50% of the cytochrome *P*-450 into cytochrome *P*-420 = 0.25 mM) than were microsomal fractions from control rats (I_{50} = 1.2 mM) or phenobarbital-pre-

Fig. 4. Effect of time and NADPH on the tetraethyltin- and triethyltin bromide-dependent conversion of cytochrome *P*-450 into cytochrome *P*-420 *in vitro*

The experiments were performed at 37°C as described in the Materials and methods section and Fig. 3, except that the time of incubation was varied. ○, ●, Tetraethyltin; △, ▲, triethyltin bromide. The experiments were performed in the absence (●, ▲) or presence (○, △) of 0.5 mM-NADPH.

Fig. 5. Concentration-dependence of triethyltin bromide-dependent formation of cytochrome P-420

The experiments were performed at 37°C for 10 min as described in the Materials and methods section and Figs. 3 and 4, except the triethyltin bromide concentration was varied. The conversion of cytochrome P-450 into cytochrome P-420 by the organometallic was monitored with liver microsomal fractions from untreated (□), phenobarbital-treated (○) and 5,6-benzoflavone-treated (△) rats. Total cytochrome recovery (●) was determined by measuring the amount of remaining cytochrome P-450 and of cytochrome P-420 formed by the method of Omura & Sato (1964).

treated rats ($I_{50} = 0.8$ mM). In all cases, the total amount of cytochrome could be accounted for as either cytochrome P-450 or cytochrome P-420. Diethyltin dibromide could also cause the formation of cytochrome P-420, but was much less effective than triethyltin bromide. Triethyl-lead bromide and tributyltin bromide were nearly as effective as triethyltin bromide. The tetraethyl-tin and -lead derivatives did not affect the conversion without addition of NADPH, and none of the germanium compounds caused the time-dependent formation of cytochrome P-420 in the absence or presence of NADPH.

Table 4 shows the effect of di- and tri-ethyltin salts on several microsomal enzyme activities. Diethyltin dibromide was only poorly effective in inhibiting cytochrome P-450-dependent metabolism of benzphetamine, ethoxycoumarin and ethoxyresorufin, and had no effect on the NAD(P)H-cytochrome *c* reductase activities of liver microsomal fractions. However, triethyltin bromide strongly inhibited metabolism of these three monooxygenase substrates. A difference in effect was seen with cytochrome *c* reductase activities. The NADPH-dependent activity was inhibited by 25–50%, but the NADH-dependent activity was stimulated. These results may be due to a direct

Table 4. Effect of di- and tri-ethyltin on several liver microsomal activities

The cytochrome *c* reductase and benzphetamine demethylase assays were performed as described in the Materials and methods section on microsomal protein samples which had been incubated in the presence of 2 mM-organotin for 5 min at 37°C, and the *O*-dealkylase assays were performed on microsomal protein samples incubated in the presence of 0.2 mM-organotin. The liver microsomal protein was obtained from rats pretreated with 5,6-benzoflavone (BF), phenobarbital (PB), or corn oil (CON). These concentrations of organometallics gave maximal inhibition in all cases. The values are averages of three experiments and had standard deviations of less than 5–10% of the average.

Assay	Animal pretreatment	Activity (% of control)
Diethyltin		
NADPH-cytochrome <i>c</i> reductase	CON	97
	BF	92
	PB	99
NADH-cytochrome <i>c</i> reductase	CON	100
	BF	100
	PB	100
Benzphetamine demethylase	CON	82
	BF	47
	PB	80
Ethoxycoumarin de-ethylase	CON	100
	BF	87
	PB	92
Ethoxyresorufin de-ethylase	CON	100
	BF	72
	PB	100
Triethyltin		
NADPH-cytochrome <i>c</i> reductase	CON	73
	BF	51
	PB	74
NADH-cytochrome <i>c</i> reductase	CON	235
	BF	203
	PB	240
Benzphetamine demethylase	CON	23
	BF	2
	PB	20
Ethoxycoumarin de-ethylase	CON	25
	BF	26
	PB	24
Ethoxyresorufin de-ethylase	CON	43
	BF	17
	PB	40

effect of the alkyltin derivative on the individual flavoprotein reductases.

Di- and tri-ethyltin bromide-induced microsomal lipid peroxidation

Because of the possible formation of free-radical intermediates during the metabolism of triethyltin bromide, the extent of lipid peroxidation that occurs

in the presence of ethyltin derivatives was determined. The rates of lipid peroxidation in the absence of the organometallic derivatives, expressed as nmol of malonaldehyde/min per mg of protein, from untreated and phenobarbital-pretreated rats were 0.03 and 0.09 respectively. Tetraethyltin caused a 2-fold increase in lipid peroxidation in the presence of microsomal protein from control or phenobarbital-treated rats, NADPH and oxygen. However, triethyltin bromide caused a 4–5-fold increase in lipid peroxidation in microsomal fractions from untreated or phenobarbital-treated rats. Diethyltin dibromide was slightly more effective (2–3-fold increase) than tetraethyltin. Animal pretreatment with phenobarbital increased organometallic-dependent lipid peroxidation; this may be due to increased metabolism of the organotin compounds. The ethylene and ethane formed by liver microsomal fractions in the presence of NADPH and oxygen was not due to lipid peroxidation, since very little ethylene and ethane were formed when tributyltin bromide was used as a substrate. In fact, significant amounts of butene and butane were formed under these conditions from the tributyltin salts. Although addition of 1000 p.p.m. of CCl_4 to microsomal protein from phenobarbital-treated rats in the presence of NADPH and oxygen resulted in marked stimulation of lipid peroxidation (0.5 nmol of malonaldehyde/min per mg of protein), the amount of ethylene produced was approx. 0.5% and 2% of that obtained from tetraethyltin and triethyltin bromide respectively. No ethane was detected under these conditions. It is notable that the order of potency in inducing lipid peroxidation *in vitro* apparently parallels the toxicity of the tin derivatives in brain.

Discussion

The oxidative metabolism of alkyl-tin and -lead compounds has been demonstrated by several research groups (Aldridge & Cremer, 1955; Bolanowska & Wisniewska-Knypl, 1971; Bridges *et al.*, 1967; Stevens *et al.*, 1961), but little information has been provided on the dealkylation products derived from the alkyl moiety which has been oxidized. Bridges *et al.* (1967) noted that one could recover all of the tin that was given as diethyltin dichloride to

rats *in vivo*, but only 50% of the ^{14}C label associated with [ethyl- ^{14}C]diethyltin dichloride could be recovered. Since that study could not account for the lost radioactivity as $^{14}\text{CO}_2$ or as an intermediary metabolite that might lead to $^{14}\text{CO}_2$, they postulated that the product might be a non-reactive volatile product such as ethane. A similar result was noted for triphenyltin acetate (Williams *et al.*, 1978), except that benzene was noted as a respired product. In addition, the metabolites of benzene in urine, phenol sulphate and quinol sulphate, were observed. More recently, it has been demonstrated that triethyl-lead produces measureable amounts of ethane and ethylene *in vivo* in vitamin E- and selenium-deficient rats (Ramstoeck *et al.*, 1980).

The results of our work on the ethyl-substituted organometallic derivatives of germanium, tin and lead indicates that the cytochrome *P*-450-dependent dealkylation of these compounds to yield ethane and ethylene also occurs. Further, the tetra-, tri- and di-ethyl derivatives of tin can be successively dealkylated to give the respective dealkylated products and the hydrocarbons. To date, no other products of the ethyl-substituted organometallics are known. The effects of animal pretreatment regimen on the product ratio shows that more than one form of the cytochrome may be involved in hydrocarbon formation. The effect of anaerobiosis on the reaction strongly suggests that ethane formation may occur by a reductive process, as well as an oxidative process which forms both hydrocarbons (Scheme 1). Such results are supported by our demonstration that sodium dithionite is involved in a chemical reaction to yield ethane and ethylene during dealkylation of triethyltin bromide. Benzene and ethane have been detected during the chemical hydrogenation of triphenyltin acetate and triethyltin acetate, respectively, at 200°C and 60 atm (6 MPa) (Neuman, 1970). The generation of ethane and benzene may occur, in part, via a radical pathway, as described by Neuman (1970). Although no evidence exists for the role of such radical intermediates in the toxicity of the organometallic compounds involved, a radical intermediate may be linked to deleterious processes, such as lipid peroxidation (Buege & Aust, 1978). Scheme 1 attempts to account for the apparent mechanisms

Scheme 1. *Postulated scheme for hydrocarbon formation from organotin salts under aerobic and anaerobic conditions*

for hydrocarbon formation from the organometallic derivatives of Group-IV elements: C-hydroxylation and one-electron reduction.

The present paper demonstrates that triethyltin salts have a profound effect on cytochrome *P*-450 and its flavoprotein reductase. Cytochrome *P*-450 is converted into cytochrome *P*-420 by triethyltin bromide in the absence or presence of NADPH. The conversion of cytochrome *P*-420 is apparently paralleled by a loss of metabolic function. It was of interest that tetraethyltin only caused cytochrome *P*-420 formation in the presence of NADPH, suggesting that the organometallic compound is converted into its triethyl derivative, which may be responsible for the loss of function of the cytochrome. This metabolic reaction must account for the lag seen in the toxic response in animals treated with tetraethyltin, and the possible destructive effect on the cytochrome may account, in part, for the slower conversion of the diethyl derivative into the ethyltin salt (Cremer, 1957, 1958; Bridges *et al.*, 1967). Interestingly, our results demonstrated that triethyltin chloride was more effective at inducing lipid peroxidation than was diethyltin dibromide *in vitro*. Tetraethyltin caused only minimal increases in peroxidation of microsomal lipids. Similar organotin-protein interactions have been seen with proteins and triethyltin salts, notably rat and cat haemoglobin (Elliott & Aldridge, 1977; Rose, 1969) and glutathione transferase B (Henry & Byington, 1976; Tipping *et al.*, 1979).

Although it is not clear whether a reactive intermediate is formed during metabolism to account for portions of the toxicity caused by the lower alkyl-organometallics, considerable evidence exists that the di- and tri-ethyltin salts differentially affect mitochondrial function as described by Aldridge (1976). This suggests that an alkyl radical cannot solely account for the toxic effects of these compounds. The significance *in vivo* of the results that the di- and tri-ethyltin bromides convert cytochrome *P*-450 into cytochrome *P*-420 *in vitro* is not clear, but it has been demonstrated that pre-treatment of animals *in vivo* with lead acetate and methylmercury chloride has deleterious effects on cytochrome *P*-450 function (Alvares *et al.*, 1972; Chou & Cornish, 1978; Kappas & Maines, 1980). Studies directed toward the effect of ethyl-substituted tin derivatives on rat hepatocyte function are required to evaluate the influence of these compounds on cytochrome *P*-450 in intact cells and on toxic processes such as lipid peroxidation or cell lysis.

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