

Bacterial Mutagenicity of Pyrolysis Tars Produced from Chloro-organic Fuels

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Droplets of toluene and three chlorinated organics, *ortho*-dichlorobenzene, 1,2-dichloroethane, and trichloroethylene, were pyrolyzed in pure nitrogen. The composition and bacterial mutagenicity of the product tars were measured. The presence of organic chlorine was found to affect both pyrolysis product tar composition and total tar mutagenicity. Pyrolysis in the absence of chlorine produced tars whose bacterial mutagenicity was found to be largely due to the presence of cyclopenta[*cd*]pyrene, fluoranthene, and benzo[*a*]pyrene. Small amounts of chlorine in the fuel (i.e., Cl/H molar ratios of less than 0.3) enhanced the formation of highly condensed polycyclic aromatic hydrocarbons (including cyclopenta[*cd*]pyrene) and increased tar mutagenicity. Larger amounts of organic chlorine (Cl/H ratios of between 0.3 and 0.6) resulted in significant yields of mono- and dichlorinated aromatics and higher levels of tar mutagenicity, which could not be accounted for by the presence of mutagens produced by pyrolysis in the absence of chlorine. Furthermore, unlike tars containing little or no chlorine, tars containing aryl chlorine were more mutagenic in the absence of added enzymes (intended to mimic *in vivo* mammalian metabolism) than in their presence. We hypothesize that at least one of the chlorinated aromatic products is strongly mutagenic. Two specific conditions that gave notably different results were *a*) the low-temperature (i.e., below 1400 K) pyrolysis of *ortho*-dichlorobenzene, which produced tri- and tetrachlorinated biphenyls almost exclusively; and *b*) the chlorine-rich pyrolysis of trichloroethylene, during which mostly perchloroaromatics were formed. Neither of these tars was found to mutate bacteria.

Introduction

Many of the polycyclic aromatic compounds (PACs) condensed as tar on the surface of soot are mutagenic (1,2) or carcinogenic (3,4). The types and amounts of these toxic combustion by-products that are produced by pyrolysis in diffusion flames are functions of the elemental and structural composition of the fuel and the conditions governing the rate and extent of fuel conversion during pyrolysis. In the case of incineration, the presence of organically bound chlorine in the waste stream can have a significant effect on pyrolysis chemistry. The pyrolysis of chloro-organics has been found to produce chlorinated aromatics, with yields dependent largely on the molecular structure of the fuel at low temperatures and on the elemental content of the fuel at high temperatures (5). Pyrolysis in a chlorine-rich environment produces perchlorocompounds (6). The presence of chlorine in fuels has also been found to promote aromatic condensation and soot formation during pyrolysis, resulting in tars that contain a large fraction of *peri*-fused aromatic structures (7).

Several classes of PACs, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated dibenzo-*p*-dioxins and dibenzofurans, have been found in the emissions of refuse incinerators burning chlorinated wastes (8-14). Increased resistance to biodegradation has been associated with increased chlorine substitution, as evidenced by the environmental persistence and accumulation of polychlorinated aromatics (15). Many of these compounds are toxic (16); vinyl chloride has been implicated as a potential causative agent of human liver cancer (17).

Few studies have directly linked incinerator emissions with health impact, however. In one epidemiological investigation that did, it was found that the frequencies of dairy cattle and human twinning increased in areas near incinerators burning municipal and chemical wastes containing chlorine (18). It was hypothesized that the presence of polychlorinated hydrocarbons, some of which have estrogenic and fertility-related properties, may account for this association; however, causality could not be established. With the limited amount of data from field studies, controlled laboratory studies using bioassays as indicators of potential health risk are needed to establish incineration guidelines and regulations.

Bacterial mutation assays represent one method for assessing overall health risk associated with a complex sample; their use has been reviewed by Lewtas (19). Recently, these assays have been used to measure the induced mutation of bacteria by products of ethylene combustion (20), coal pyrolysis (21), municipal waste incineration (22-24), and agricultural waste incineration (25,26). Biological activity, like chemical reactivity, is a function

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of molecular structure. It has been shown that factors governing the biological behavior of PACs include the size and configuration of the parent aromatic molecule (27,28) and the presence or absence of heteroatoms or substituent groups (29–34). Further complicating matters, the frequency of mutation is also dependent on the set of catalysts (enzymes) that are present in an exposed cell. In the human body, this is best considered an idiosyncratic set for each cell (tissue) type. Bacteria have their own set of metabolizing enzymes, providing one measure of induced mutation when exposed to chemicals. Enzymes can also be added to cell cultures in bioassay experiments in an attempt to mimic specific types of mammalian metabolism.

Although the pyrolytic transformation of fuel to products results in a large increase in the number of effluent chemicals, it is generally believed that the number of mutagens contributing significantly to the total mutagenicity is small. Of PAHs formed during the high-temperature combustion of hydrocarbons, the major mutagens that have been found are cyclopenta[*cd*]pyrene (CPP), fluoranthene, and benzo[*a*]pyrene. Little is known, however, about the mutagenicity of pyrolysis products of chlorinated organics. DeMarini et al. (23) found that the mutagenic potency of rotary kiln emissions, as measured by a bacterial mutation assay with metabolic activation via the addition of mammalian enzymes, was reduced by the presence of polyvinylchloride and carbon tetrachloride in the feed stock. The chemical composition of the chlorinated organic emission fraction was not measured, however. In analysis of organic emissions of two municipal waste incinerators burning polyethylene plastic, DeMarini et al. (24) found mutagenic activity to be greater in the absence of mammalian enzymes than in their presence. Furthermore, they found that the moderately polar fraction of the organic emissions was responsible for the mutagenic activity. As in the rotary kiln study, a detailed chemical analysis of the emissions was not reported.

In this paper, effects of chlorine on pyrolysis product pathways are reviewed; these results have been reported elsewhere (5–7). We then discuss measurements of bacterial mutation induced by the pyrolysis tar extracts, comparing them with the total contribution of all identified mutagens. Based on this comparison, we speculate on the presence of new mutagens in the chloro-organic pyrolysis tars. We also consider the effects on mutagenicity of the enhanced formation of *peri*-fused PAH mutagens during pyrolysis of chlorinated organics and the presence of chlorinated arenes in the tars.

Methods

Reactor and Sampling Apparatus

Streams of monodisperse droplets, generated by a vibrating orifice device, were injected down the center of a laminar flow, isothermal drop-tube reactor in an environment of pure nitrogen. A metered stream of droplets, 40 μm in diameter and 2.5 droplet diameters in spacing, was injected coaxially with nitrogen, preheated to furnace wall temperatures ranging from 1100 to 1500 K. Average gas residence times of between 1.1 and 1.5 sec were maintained, sufficient for complete vaporization of the fuel droplets. A collection probe with gas quench and wall purge was used to channel the entire product stream through a 0.2 μm pore,

mesh filter at ambient temperature. Tar yield was measured gravimetrically, after extraction from the filter sample by solubility in dichloromethane (DCM). Soot, defined here as the DCM-insoluble fraction, was determined by difference. This equipment and these procedures are described in greater detail elsewhere (5–7).

Toluene, three chlorinated organics, and equal volume mixtures of toluene and the chlorinated organics were pyrolyzed. The chlorinated compounds were *ortho*-dichlorobenzene (*o*-C₆H₄Cl₂), 1,2-dichloroethane (1, 2-C₂H₄Cl₂), and trichloroethylene (C₂HCl₃). Selection of these compounds was based on their physical properties (e.g., viscosity, boiling point, and density) being similar to those of toluene and their composition and structure allowing for the study of effects of chlorine type (aliphatic versus aromatic) and amount (chlorine-to-hydrogen molar ratios ranging from 0 to 3).

Analytical Chemistry Instrumentation

The tar fractions were subjected to the following analyses: gas chromatography (GC) with flame ionization detection (FID), with mass spectroscopy (MS) and with Fourier transform infrared detection (FTIR), and high-performance liquid chromatography (HPLC) with ultraviolet-visible (UV-vis) spectrometric detection. All of the GC work was performed on Hewlett-Packard Model 5890A systems with Quadrex methyl (5% phenyl) silicone fused-silica open tubular columns, using a helium gas mobile phase with identical flow and temperature-ramping protocol. This allowed for easy cross-referencing of spectral data by GC retention time. The HPLC analysis was performed on a Hewlett-Packard Model 1090 system with a 190 to 600 nm diode array detector and a Vydac reverse-phase column.

Bacterial Mutation Assay

Forward-mutation assay to 8-azaguanine resistance in *Salmonella typhimurium* strain TM677 was used to measure the mutagenicities of total tar samples, as described previously (35,36); a brief summary is given here. The solvent containing the pyrolysis tar product sample was exchanged from dichloromethane (DCM) to dimethyl sulfoxide (DMSO). These test samples, at concentrations ranging from 10 to 300 $\mu\text{g}/\text{mL}$, were then exposed to exponentially growing bacteria in the presence and in the absence of 5% (v/v) Aroclor induced postmitochondrial supernatant (PMS). After 2 hr, aliquots of the cell cultures were plated in the presence and in the absence of the selective agent (8-azaguanine, 50 $\mu\text{g}/\text{mL}$). Two independent cultures were used for each treatment point.

Colonies were counted after 48 hr. The mutant fraction was determined as the number of colonies formed under selective conditions divided by the number of colonies formed under non-selective conditions. If this ratio was larger than that found for simultaneous untreated control cultures ($n = 2$) with greater than 99% confidence, and if the ratio also exceeded the 95% upper confidence limit of the mutant fraction for the cumulative historical control ($n \geq 1000$), the test was considered positive. The induced mutant fraction was calculated by subtracting the background mutation level. Induced mutant fraction measurements are presented in this paper at a dose of 30 $\mu\text{g}/\text{mL}$.

Results and Discussion

Tar Product Composition

Toluene pyrolysis yielded a broad distribution of PAHs, with structures ranging in aromatic carbon number from 10 to 26. These compounds were formed by carbon-fragment addition, aryl-aryl recombination, condensation, and isomerization. At high conversion of fuel to tar and soot (i.e., at high temperatures), PAHs consisting of four and five fused rings were found to be most abundant. The presence of organically-bound chlorine was found to affect pyrolysis product composition in two ways: by the formation of chlorinated aromatics and by the promotion of aromatic condensation and soot formation. At high temperatures, little chlorine was found in the aromatic tars produced by the pyrolysis of fuels with Cl/H molar ratios of less than 0.3; most of the aryl chlorine that was detected in these tars was bound to unfused benzene rings. In pyrolysis of fuels with higher chlorine contents ($0.3 < \text{Cl}/\text{H} < 0.6$), the product tars contained significant quantities of mono- and dichlorinated aromatics. With regard to aromatic structure, the formation of *peri*-fused aromatics, including CPP, was favored by increased chlorine content over the formation of *ortho*-fused structures and biaryls. Only trace amounts (less than 0.5 mole % of the total tar yield) of new structures were detected in the tars from chlorinated hydrocarbon pyrolysis; these consisted mostly of highly condensed structures with ethynyl substituents.

Two sets of conditions that were studied gave notably different tar product distributions. Low-temperature pyrolysis of *o*-C₆H₄Cl₂ (temperatures less than 1400 K) yielded tri- and tetrachlorinated biphenyls almost exclusively (5). In the absence of ring rupture, carbon growth appeared to occur entirely by aryl dimerization. Above 1400 K, fragmentation occurred, and a broad distribution of products, similar to those described in the preceding paragraph, was found. A second condition that produced a unique product distribution was the pyrolysis of a chlorine-rich fuel, C₂HCl₃. Here, mostly perchloroaromatics were found (6). Carbon growth in this system was limited, with no identified structure containing more than 14 carbon atoms.

Mutagenicity with Postmitochondrial Supernatant

The mutagenicities of 30 $\mu\text{g}/\text{mL}$ doses of product tars in the presence of mammalian enzymes (i.e., +PMS) are shown as a function of pyrolysis temperature in Figure 1. As a reference point, the induced mutant fraction of pure benzo[*a*]pyrene at 30 $\mu\text{g}/\text{mL}$ is about 90×10^{-5} . All of the tars that contained a broad distribution of aromatic products (i.e., all tars from pyrolysis of toluene-containing fuels, as well as tars from 1,2-C₂H₄Cl₂ pyrolysis and high temperature *o*-C₆H₄Cl₂ pyrolysis) were mutagenic. The tars containing a narrow distribution of products, namely, those produced by pure C₂HCl₃ pyrolysis (containing predominantly perchloro compounds) and those produced by low temperature pyrolysis of *o*-C₆H₄Cl₂ (composed almost exclusively of PCBs), were not mutagenic.

Six PAHs that are known bacterial mutagens were detected in significant abundance (greater than 1 mole %) in these tars. In order of decreasing potency, these PAH mutagens are CPP, fluoranthene, benzo[*a*]pyrene, acephenanthrylene, aceanthrylene, and cyclopenta[*hi*]acephenanthrylene. Five of these

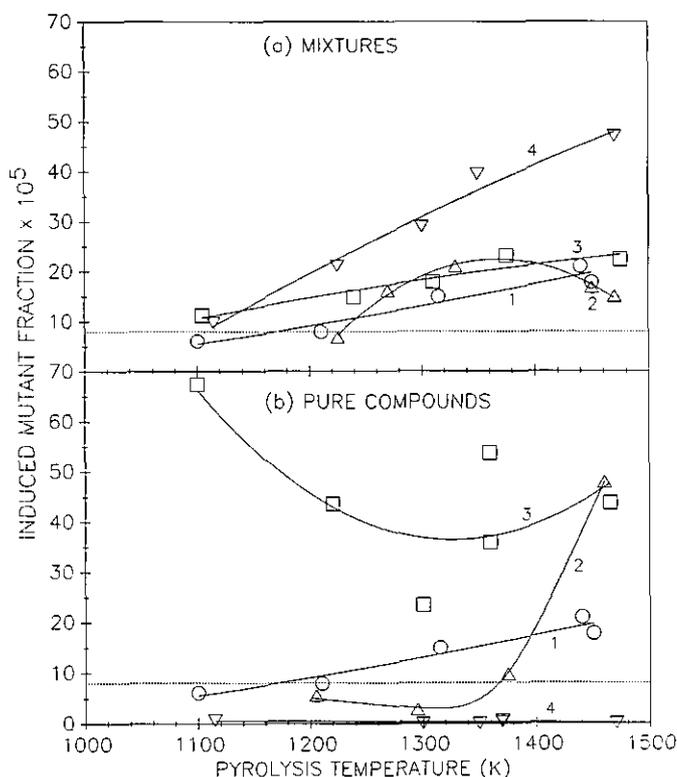


FIGURE 1. Bacterial mutagenicity (+postmitochondrial supernatant) of pyrolysis tar samples, measured for a 30- $\mu\text{g}/\text{mL}$ dose. The dotted lines in the graphs indicate the 99% confidence limit that the sample is mutagenic in this assay. The top graph contains plots of data on pyrolysis tars of pure toluene and binary mixtures of toluene and three chlorinated organics. The bottom graph contains plots of data on tars from pure compound pyrolysis. (1) toluene; (2) *o*-C₆H₄Cl₂; (3) 1, 2-C₂H₄Cl₂; (4) C₂HCl₃.

were detected in the toluene pyrolysis tars; their relative yields are given in Table 1. Also shown are the relative potencies of these compounds as bacterial mutagens. The total mutagenicity of the toluene pyrolysis tar increased as temperature increased, as did the yields of the five mutagens. Assuming additivity, mutagenicity is determined by the sum of the product of the potency and yield of each tar constituent. Mutagen additivity in each of the toluene pyrolysis tars accounts for the total mutagenicity measurement to within 25%. CPP, fluoranthene, and benzo[*a*]pyrene appear to be responsible for about 90% of the biological activity.

Table 1. Major contributors to mutagenicity in toluene pyrolysis tars.

Mutagen	Relative potency ^a	Yield, mole %			
		Temperature, K			
		1100	1210	1315	1440
Cyclopenta[<i>cd</i>]pyrene	2.5	0.09	0.91	1.8	3.4
Fluoranthene	1.0	6.4	4.0	5.3	9.2
Benzo[<i>a</i>]pyrene	1.0	0.00	1.2	2.1	2.5
Acephenanthrylene	0.25	2.1	2.5	2.9	3.9
Aceanthrylene	0.12	0.68	2.1	2.8	2.9
Sum of mutagen contributions ^b $\times 10^5$		6.5	7.5	11.7	19.3
Measured mutagenicity (total) $\times 10^5$		6.1	7.9	14.9	21.0

^aInduced mutant fraction of benzo[*a*]pyrene at 30 $\mu\text{g}/\text{mL}$ = 90×10^{-5} .

^bMutagenicity = potency \times yield.

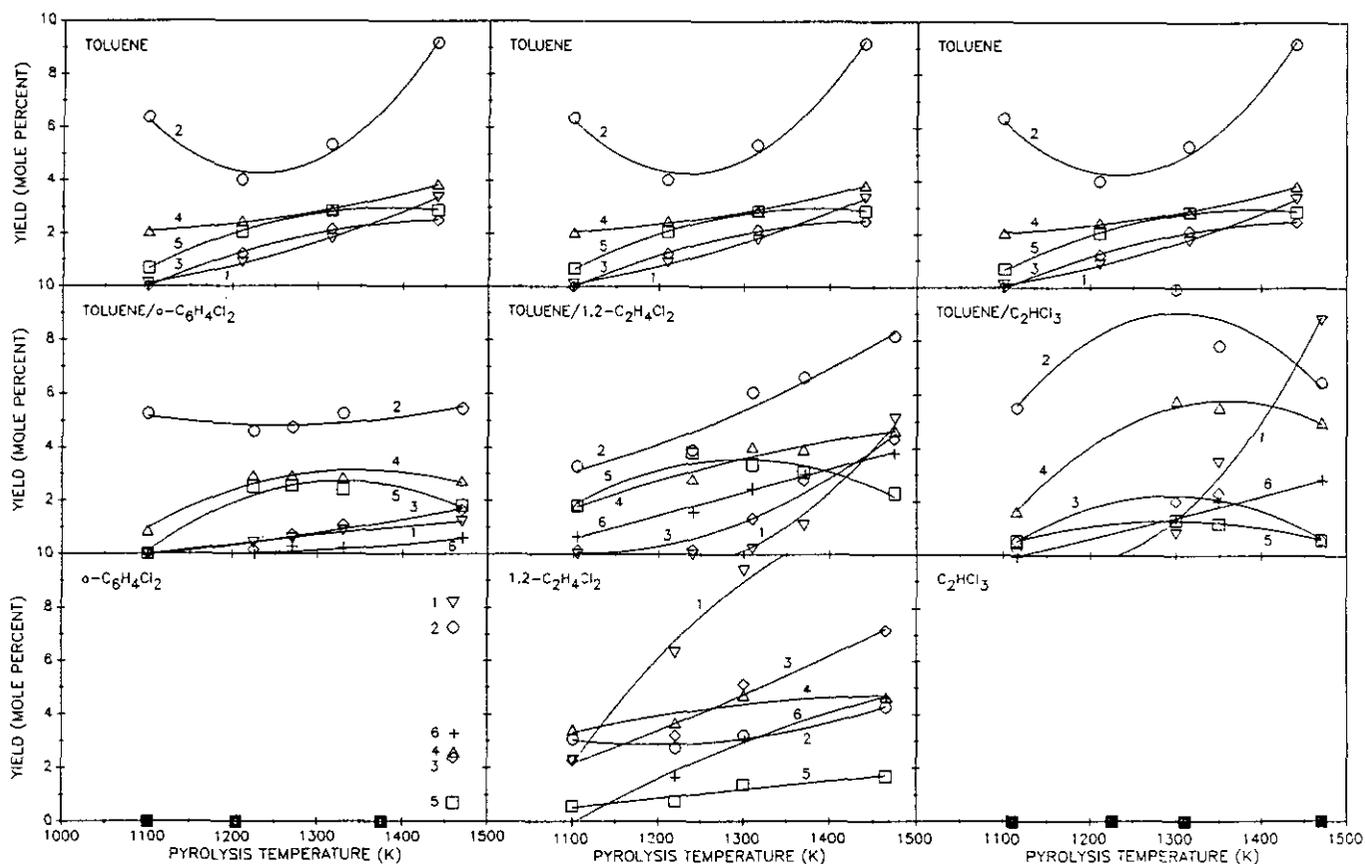


FIGURE 2. Yields, as percentages of the total tar yield, of six chemicals that are known mutagens formed by the pyrolysis of pure toluene (top), binary mixtures of toluene and three chlorinated organics (middle), and pure chlorinated organics (bottom). Chemicals (in order of decreasing mutagenic potency) are: (1) CPP; (2) fluoranthene; (3) benzo[*a*]pyrene; (4) acephenanthrylene; (5) acanthrylene; (6) cyclopenta[*hi*]acephenanthrylene.

Yields of the six PAH mutagens identified in the tars from pyrolysis of each fuel, over all of the temperatures studied, are shown in Figure 2. Adding *o*-C₆H₄Cl₂ to toluene had little effect on the product distribution and mutagenicity of the resulting pyrolysis tars (Fig. 1a). Pyrolysis of the 1,2-C₂H₄Cl₂/toluene mixture yielded tars with slightly increased mutagenicity (10–50%). Adding C₂HCl₃ to toluene produced pyrolysis tars of significantly higher mutagenicity (50–200%). In general, the yields of known mutagens increased with increasing pyrolysis temperature, consistent with the measurements of total tar mutagenicity. This was particularly true of CPP, the most potent mutagen found in these tars. As discussed elsewhere (7), CPP formation is promoted in environments that favor the formation of *peri*-fused aromatics, i.e., pyrolysis at high temperatures and in the presence of chlorine. Thus, the measurements of potential health impact by the bacterial mutation assay appear to be best correlated with the presence of CPP.

The contributions of known PAH mutagens do not appear to account for all of the measured mutagenicity in several of the pyrolysis tars, however. Pure *o*-C₆H₄Cl₂ pyrolysis at 1460 K, pure 1,2-C₂H₄Cl₂ pyrolysis at low temperatures and pyrolysis of the C₂HCl₃/toluene mixture at high temperatures yielded very mutagenic tars in which the summed contributions of known PAHs do not appear to account for the measured levels (Table 2). Each of these tars was composed of a broad distribution of

aromatics, including large amounts of partially chlorinated aromatics. We hypothesize that at least one unidentified mutagen is present in the tars from pyrolysis of chlorinated organics under the conditions specified above. It is possible that a mutagen not produced during pure hydrocarbon pyrolysis was formed either by a chloro-organic-dependent pathway to an unidentified PAH mutagen or by the addition of chlorine atom to an aromatic structure. We speculate that it was the latter mechanism because these tars contained no PAHs in more than trace amounts that were not found in the pure toluene pyrolysis tars. Furthermore, each of these tars contained significant amounts of mono- and dichlorinated congeners of the major PAH mutagens.

Data that support this speculation are shown in graph form in Figure 3. Pyrolysis of 1,2-C₂H₄Cl₂ at low temperatures produced highly mutagenic tars containing very small amounts of the major PAH mutagens and large amounts of mono- and dichlorinated aromatics. As pyrolysis temperature was increased, the difference between the measured total tar mutagenicity and the sum of the PAH mutagen contributors decreased. The yields of chlorinated arenes also decreased. Similar correlations between chlorinated aromatic yields and unaccounted-for mutagenicity were obtained for the other tars studied.

Pyrolysis of *o*-C₆H₄Cl₂ at low temperatures yielded tars containing only PCBs and having no significant mutagenicity. Pyrolysis of pure C₂HCl₃ produced nonmutagenic tars containing

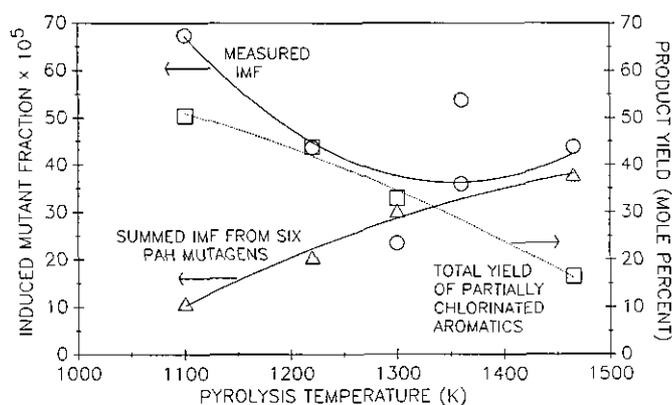


FIGURE 3. Comparison of the total measured bacterial mutagenicity ($30 \mu\text{g}/\text{ml}$ dose) of the 1,2-dichloroethane pyrolysis tars and the sum of the individual bacterial mutagenicity contributions of the six PAHs listed in Table 1. The total yield of partially chlorinated aromatics having molecular formulas $\text{C}_{14}\text{H}_9\text{Cl}$, $\text{C}_{14}\text{H}_8\text{Cl}_2$, $\text{C}_{16}\text{H}_9\text{Cl}$, $\text{C}_{16}\text{H}_8\text{Cl}_2$, $\text{C}_{18}\text{H}_9\text{Cl}$, $\text{C}_{18}\text{H}_{11}\text{Cl}$ and $\text{C}_{20}\text{H}_{11}\text{Cl}$ is also shown.

perchloroaromatics. Thus, under special conditions, the presence of chlorine reduces tar mutagenicity, as assessed by the bacterial mutation assay. This finding is consistent with the report by DeMarini et al. (23) on the incineration of polyvinylchloride and carbon tetrachloride in rotary kiln. It is noted, however, that these results provide only one measure of potential

health risk; conclusions about overall health risks associated with these tar products should be drawn from the large body of information available in the literature on the health impacts from exposure to compounds such as PCBs and hexachlorobenzene.

Mutagenicity without Postmitochondrial Supernatant

The bacterial mutagenicities of selected tar samples were measured without addition of mammalian enzymes (i.e., -PMS). One tar from pyrolysis of each of the pure liquids was analyzed; dose-response curves for both the +PMS and -PMS tests are presented in Figure 4. The tar from pure toluene pyrolysis was a more potent +PMS mutagen than a -PMS mutagen. This is consistent with other data indicating that unsubstituted PAHs are generally inactive in -PMS tests. Tars from pyrolysis of the three chlorinated organics each contained more potent -PMS mutagens than +PMS mutagens. These tars contained significant amounts of chlorinated arenes. We speculate that chlorine substitution is responsible for the -PMS mutagenicity, consistent with the finding of DeMarini et al. (24) that moderately polar compounds appear to be more potent mutagens in the absence of mammalian enzymes.

Even in the two cases in which +PMS mutagenicity was negligible (i.e., the case of C_2HCl_3 pyrolysis at 1370 K yielding perchloroaromatics and the case of $o\text{-C}_6\text{H}_4\text{Cl}_2$ pyrolysis at 1295 K yielding PCBs), the -PMS mutagenicity was significant. In the

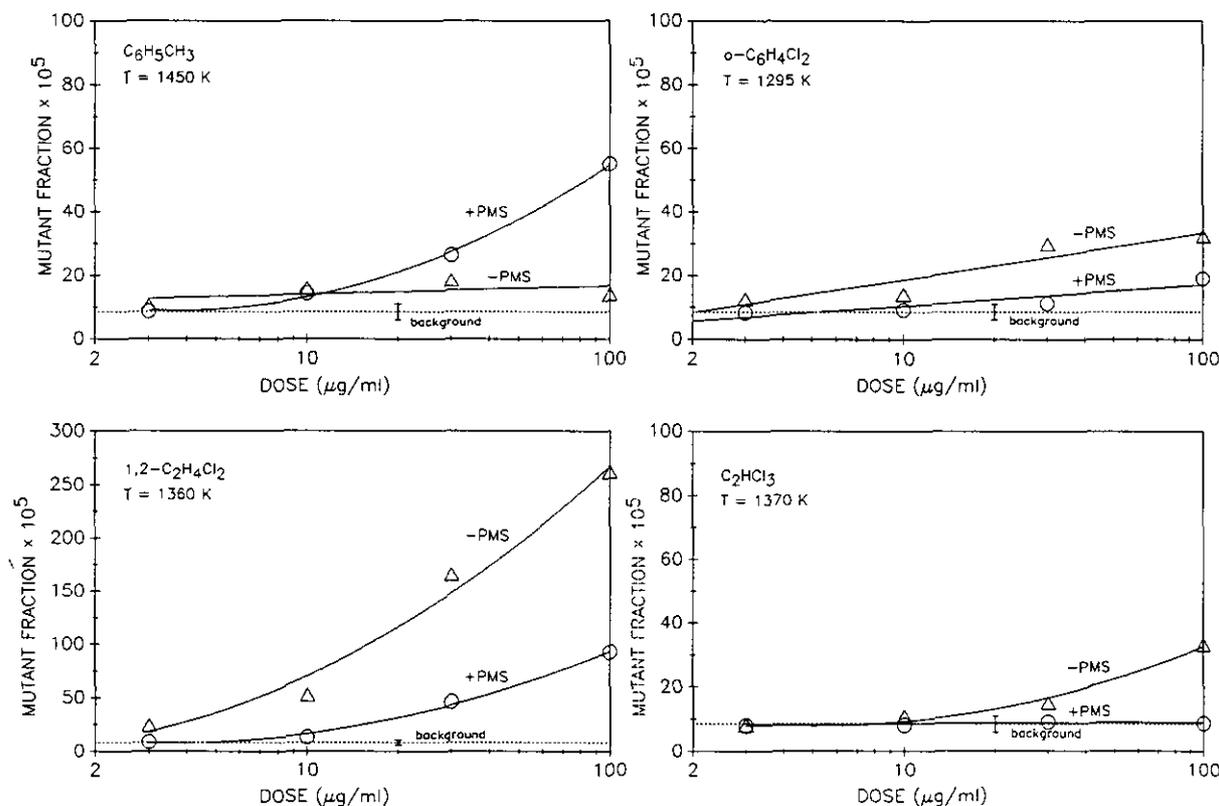


FIGURE 4. Dose-response bacterial mutagenicity (with and without postmitochondrial supernatant [PMS]) of product tars from pyrolysis of toluene, $o\text{-C}_6\text{H}_4\text{Cl}_2$, $1,2\text{-C}_2\text{H}_4\text{Cl}_2$, and C_2HCl_3 . The dotted lines indicate the background mutant fraction that was measured. Note the increased scale of the ordinate axis in the $1,2\text{-C}_2\text{H}_4\text{Cl}_2$ plot.

Table 2. Comparison of measured induced mutant fraction with contributions from known PAH mutagens in tars from organo-chlorine pyrolysis.

Compound	Temperature,		IMF _{meas} ^b
	K		
<i>o</i> -C ₆ H ₄ Cl ₂ /toluene	1470	10.0	15.0
	1330	8.6	21.0
	1270	7.3	16.1
	1225	6.2	6.8
	1100	4.9	—
1,2-C ₂ H ₄ Cl ₂ /toluene	1475	24.5	22.4
	1375	12.5	23.1
	1310	8.7	17.9
	1240	4.9	14.9
	1105	3.8	11.3
C ₂ HCl ₃ /toluene	[1470	27.6	47.1] ^c
	[13.50	18.6	39.5] ^c
	[1300	14.3	29.0] ^c
	1225	—	21.1
	1115	6.1	9.8
Pure <i>o</i> -C ₆ H ₄ Cl ₂	[1460	28.1	47.9] ^c
	1375	0.0	9.6
	1295	0.0	2.8
	1205	0.0	5.5
	1100	0.0	—
Pure 1,2-C ₂ H ₄ Cl ₂	1465	37.8	43.8
	1370	—	46.0
	1300	30.2	23.4
	[1220	20.5	43.6] ^c
	[1100	10.8	67.4] ^c
Pure C ₂ HCl ₃	1470	0.0	< 0.0
	1370	0.0	0.3
	1300	0.0	< 0.0
	1225	0.0	—
	1115	0.0	0.7

^aTotal induced mutant fraction from six major PAH mutagens detected in these tars.

^bInduced mutant fraction measured for pyrolysis tar samples.

^cBrackets denote tars in which additivity of known mutagens does not appear to account for the observed total tar mutagenicity.

case of 1,2-C₂H₄Cl₂ pyrolysis at 1360 K, the +PMS mutagenicity was high, and the -PMS mutagenicity was even higher. More work is needed to isolate and identify the specific compound(s) responsible for this mutagenicity.

Conclusions

The composition and bacterial mutagenicity of pyrolysis product tars were found to be affected by the presence of organic chlorine. In the absence of organic chlorine, bacterial mutagenicity was found to be largely due to the presence of CPP, fluoranthene, and benzo[*a*]pyrene in the product tars. Small amounts of chlorine (Cl/H < 0.3) enhanced the formation of *peri*-fused PAHs, including CPP, and increased tar mutagenicity. Larger amounts of chlorine (0.3 < Cl/H < 0.6) resulted in significant yields of mono- and dichlorinated aromatics and increased levels of tar mutagenicity. This mutagenicity could not be accounted for by the presence of mutagens found in the pyrolysis tars of unchlorinated fuels (e.g., CPP) and appears to be related to the presence of chlorinated aromatics. Furthermore,

unlike the tars containing little or no chlorine, the tars containing chlorine were more mutagenic in the absence of PMS than in the presence of PMS. Therefore, we hypothesize that these tars contain at least one unidentified mutagen, and we speculate that it is a mono- or dichlorinated aromatic.

Two conditions that gave notably different results are the low-temperature pyrolysis (i.e., at temperatures below 1400 K) of *o*-C₆H₄Cl₂, which produced tri- and tetrachlorinated biphenyls almost exclusively, and the chlorine-rich pyrolysis of C₂HCl₃, during which mostly perchloroaromatics were formed. Neither of these tars was found to mutate bacteria.

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